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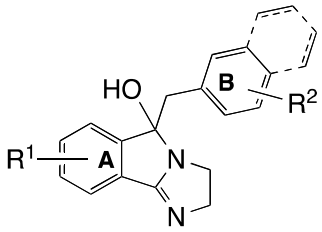
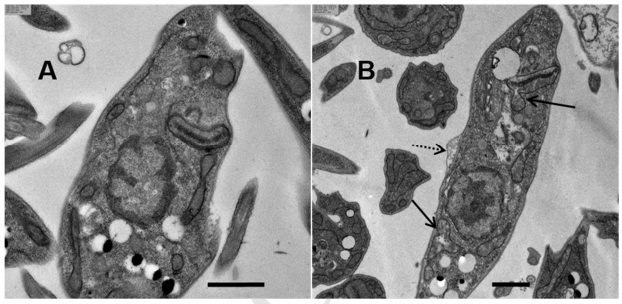
Graphical Abstract



The imidazo[2,1-*a*]isoindolol (IIOL) structure present in compounds damaging *Leishmania donovani* membrane

Micrographs of *L. donovani* promastigotes



Control parasites After adding IIOL **29**

Imidazo[2,1-*a*]isoindole scaffold as an uncharted structure active on *Leishmania donovani*

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Abstract

The human protozoan parasites *Leishmania donovani* and *L. infantum* are the causative agents of visceral leishmaniasis, as such, responsible for approximately 30,000 deaths annually. The available chemotherapeutic treatments are reduced to a few drugs whose effectiveness is limited by rising drug resistance/therapeutic failure, and noxious side-effects. Therefore, new therapeutic hits are needed. Compounds displaying the imidazo[2,1-*a*]isoindole skeleton have shown antichagasic, anti-HIV, antimalarial and anorectic activities. Here, we report the leishmanicidal activity of thirty one imidazo[2,1-*a*]isoindol-5-ol derivatives on promastigotes and intracellular amastigotes of *L. donovani*. Eight out of thirty one assayed compounds showed EC_{50} values ranging between 1 to 2 μM with selectivity indexes from 29 to 69 on infected THP-1 cells. Six compounds were selected for further elucidation of their leishmanicidal mechanism. In this regard, compound **29**, the imidazoisoindolol with the highest activity on intracellular amastigotes, induced an early decrease of intracellular ATP levels, as well as mitochondrial depolarization, together with a partial plasma membrane destructuration, as assessed by transmission electron microscopy. Consequently, the inhibition of the energy metabolism of *Leishmania* plays an important role in the leishmanicidal mechanism of this compound, even when other additional targets cannot be ruled out. In all, the results supported the inclusion of the imidazoisoindole scaffold for the development of new leishmanicidal drugs.

Keywords: Imidazoisoindolols, antileishmanial activity, energy metabolism.

1. **Introduction**

Leishmaniasis is a group of diseases caused by protozoan parasites from over 20 species of the genus *Leishmania*, transmitted to humans by the bite of infected female phlebotomine sandflies. It is considered as the third-most-common parasitic disease after schistosomiasis and malaria on the basis of its morbidity and disability-adjusted life years (DALYs) [1]. With an estimated 700,000 to 1 million new cases and some 26,000 to 65,000 deaths occurring every year, leishmaniasis continues to be a menace in a large number of countries worldwide [2]. From a clinical perspective, leishmaniasis is grouped under three major forms. The cutaneous leishmaniasis is its mildest form, producing ulcers at the site of the bite; the mucocutaneous form courses with lymphatic migration of *Leishmania* from the initial bite into mucosal tissues of the nasal, oral and throat cavities, leading to destruction of the cartilaginous tissue. Nevertheless, the most severe form of the disease is the visceral leishmaniasis, caused by *Leishmania donovani* and *L. infantum* (also known as *L. chagasi*). In VL the parasite infects mononuclear phagocyte cells in liver, spleen and bone marrow, causing the visceral form of leishmaniasis (VL) or kala-azar, fatal in more than 95% of cases if left untreated. It is characterized by irregular episodes of fever, weight loss, hepatosplenomegaly and anaemia. VL is endemic in the Indian subcontinent and East Africa; and it is estimated that between 50,000 and 90,000 new cases of VL occur in the world every year.

Treatment options for VL include the use of pentavalent antimonials, amphotericin B (AmB), paromomycin and miltefosine [1]. Most of these drugs display several drawbacks such as low efficacy, severe side effects, parental administration, and emergence of drug resistance, or significant rate of therapeutic failure [3]. For example, miltefosine, the only oral drug for leishmaniasis treatment produces teratogenic effects, and severe gastrointestinal side effects have been reported [4].

During the last decades, substantial efforts have been invested to discover more efficacy therapeutic agents against leishmaniasis [1, 5], fuelled by the significant increase in therapeutic failure and drug resistance. Research has been focused either on the quest for natural products [6, 7], target-based drug discovery approaches encompassing, among others, nuclear [8] and kinetoplast topoisomerases [9], trypanothione reductase (TryR) [10], and biosynthetic pathways for phospholipids and sterols [11], or new synthetic molecules assayed for phenotypic output [12, 13]. Recently, nitroaromatic drugs like fexinidazole, activated by parasite nitroreductases have been assayed as new leishmanicidal drugs [14]. Nowadays, Drugs for Neglected Diseases initiative acts as the best hub for new scaffolds under the last preclinical steps of development or ongoing clinical

trials. The oxaborole DNDI-6148, the nitroimidazole DNDi-0690, the aminopyrazole DNDI-5561, and the GSK3186899/DDD853651 (*N*-(trans-4-((3-isobutyl-1H-pyrazolo[3,4-*d*]pyrimidin-6-yl)amino)-cyclohexyl)-1- phenylmethanesulfonamide 4-trans) are currently in its portfolio [5,15].

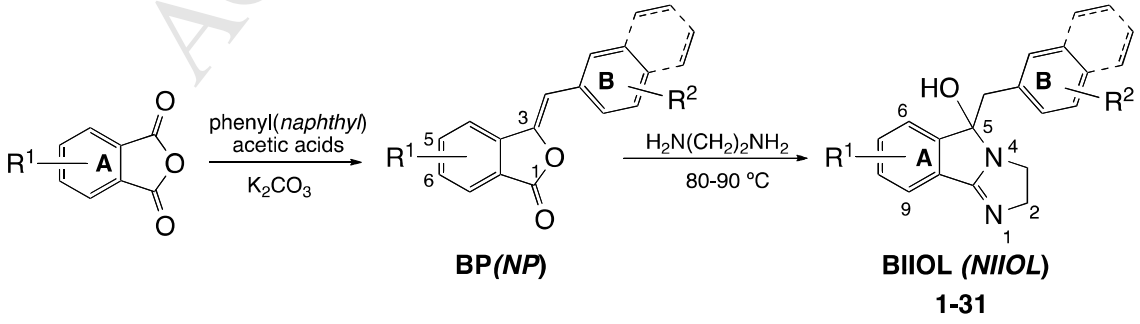
In a previous study, we synthesized and tested *in vitro* against *Leishmania spp*. some families of heterocyclic compounds sharing the dihydrostilbene scaffold, including 4-benzylidenephthalazin-1-ones, phenacetyl-benzamides, 5-benzylimidazo[2,1-*a*]isoindol-5-ols and 6-benzylpyrimido[2,1-*a*]isoindol-6-ols [16]. From these, the last two groups showed a promising leishmanicidal activity, equivalent to reference clinical drugs. To gain further insight into the leishmanicidal potential of the 5-benzylimidazo[2,1-*a*]isoindol-5-ol family, new compounds were designed, synthesized and assayed (compounds **1-31**, Scheme 1). The set of new compounds explore the chemical space of this scaffold, with variation of the electronic nature and size of the substituents, as well as their positions in the aromatic rings.

In all, we have provided a proof-of-concept for the potentiality of developing new antiparasitic drugs of the imidazoisoindolol (IIOL) scaffold, until now scarcely studied in medicinal chemistry, as well as an insight of the molecular basis underlying their antiparasitic potential.

2. **Results and Discussion**

2.1 *Chemistry*

The imidazoisoindololols (IIOLs) were obtained, by total synthesis through the route depicted in Scheme 1, as previously reported [16]. Briefly, the synthesis starts by condensation of phthalic anhydrides with substituted phenyl or naphthylacetic acids to provide benzalphthalides (BPs) and naphthylmethylphthalides (NPs), through an adaptation of the Nokihara method [17], using toluene and a Dean-Stark separator to remove the water under inert atmosphere. The resulted BPs (*NPs*) were treated with ethylenediamine at 80-90 ºC, to give the benzyl(*naphthylmethyl*)imidazo[2,1-*a*]isoindol-5-ol derivatives BIIOLs (*NIIOLs*), compounds **1-31**. Physicochemical, MS and NMR spectral data for all the new compounds are here reported.



Scheme 1. General procedure for the synthesis of 5-benzyl(*naphthylmethyl*)imidazo[2,1-*a*]isoindol-5-ol derivatives

The ^{1}H-NMR spectra of BIIOLs (*NIIOLs*) showed four multiple signals at δ 2.77/3.21 ppm and δ 3.70/4.14 ppm (imidazoline protons). The benzylic methylene signals appeared as an AB system δ 3.10 and 3.38 ppm (J = 13.6-14.0 Hz). The signal corresponding to the hydroxyl proton resonated as a singlet at δ ~ 4.20 ppm (D_2O exchangeable). The signals for aromatic protons appeared at δ 6.81 - 7.55 ppm, depending on the substituents at rings A and B. The ^{13}C-NMR spectra of BIIOLs (*NIIOLs*) showed signals for three methylene carbons at δ 39.5, 41.7 (C-2, C-3) and 44.0 ppm (benzylic methylene), for a single non protonated oxygenated carbon at δ 89.4 ppm (C-5); and for one amidine carbon around δ 167.8 ppm. The aromatic carbon signals appeared at δ between 122.2-150.2 ppm, depending on the substitution pattern of the rings.

Those compounds monosubstituted at ring A (**3** to **20**, **21** to **24** and **30** to **31**) were obtained and evaluated as mixtures 1:(0.9-0.6) of 7(8)- or 6(9)- substituted derivatives. Both regioisomers were properly identified and characterized by their ^{1}H and ^{13}C-NMR spectra and, in some cases, supported by conducting 2D experiments as COSY, HSQC and HMBC NMR correlations.

2.2 *Biological analysis*

The imidazoisoindole core remains an almost fully uncharted structure in Medicinal Chemistry. Mazindol, an imidazoisoindole with non-amphetamine anorectic properties, was withdrawn from the market due to side effects as pulmonary arterial hypertension, also found in other anorexic drugs [18]. Five additional works from Olmo *et al*. rescued the imidazo[2,1-*a*]isoindolol scaffold as the basis of antiplasmodial [16, 19], anti-chagasic [20], and anti-HIV [21] activities of heterocyclic compounds related to stilbenoids, as well as other work unveiling the leishmanicidal activity of this scaffold on *Leishmania* promastigotes [22].

In this work a double objective was pursued, first to broaden the knowledge on the structure-activity relationship for the imidazo[2,1-*a*]isoindolols on *Leishmania*, and secondly, to get insight into their leishmanicidal mechanism. For those purposes, new structures were synthesized and added to some of the compounds tested on *Leishmania* promastigotes in the previous work [22], resulting in a final set of 31 compounds. The chemical space was explored by the diversity of substituents employed, both in their chemical nature and in their positions in rings A and B of the IIOL scaffold. In order to get a closer appraisal on the chemotherapeutical potential of these molecules, they were assayed on intracellular amastigotes, as the most relevant clinical form of the parasite, and also the closest *in vitro* model for human *Leishmania* infection. Secondly, the leishmanicidal mechanism of the most potent compounds was explored, with a special focus on the

energy metabolism of the parasite; not only as a feasible ultimate target of these molecules on *Leishmania*, but also to monitor variations in parasite viability.

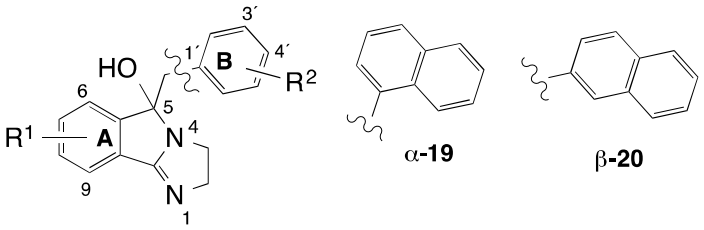
As aforementioned, in a previous work we described the leishmanicidal activity of compounds with various heterocyclic skeletons on promastigotes of *Leishmania braziliensis*, *L. amazonensis*, and *L. donovani*. There, those compounds with an imidazoisoindole skeleton without substitution on ring A, combined with nil or a single *para*-chloro substitution on ring B, showed the highest potency [22]. Therefore, they were taken as reference for the current study, and named here as compounds **1** and **2**.

The EC_{50} values on intracellular amastigotes of **1** and **2** were compared with those from compounds **3** to **31**. Eighteen of these new compounds (**3** to **20)** have a methyl group (R^1) at position 7 or 8 on ring A. This molecular trait is combined with modifications on ring B, consisting on larger aromatic groups (α- or β-naphthyl), or groups with effect on the electron density of the ring, either as electron-donor (Me, OMe, OH) or electron-attracting (F, Cl) substituents. Compound **3**, without substitution at ring B, was the internal control of this series. All these compounds were tested for leishmanicidal activity as a mixture of regioisomers 1:(0.9-0.6). The activity data for the full set of compounds against *L. donovani* intracellular amastigotes *in vitro* were compiled in Table 1.

Compound **2** showed a good inhibitory activity (EC_{50} = 2.1 μM, for amastigotes) together with an appealing selectivity index versus THP-1 cells (SI = 69.1). Compound **1**, without substitutions in rings A or B is ten times less potent than **2,** and three times less than **3**, with only a single methyl group in ring A. Besides, the SI of compound **3** was four times lower than that of **2**. The introduction of an electron-attracting substituent (Cl, F) at *ortho* or *para* positions had opposite effects, depending on the halogen. The chloro-substitution, either at position *ortho* (compound **4**, EC_{50} = 1.8 μM) or *para* (compound **7**, EC_{50} = 1.4 μM) improved slightly the activity of the parental **2**. In contrast, the fluoro derivative **6** (EC_{50} = 5.7 μM), was two and a half times less potent, and its SI halved respect to compound **2**. The introduction of either a strong electron-attracting (NO_2, **5**) or donating groups (OH, **8**; OMe, **9**) impaired their leishmanicidal activity. The sulfanyl derivative **10** (EC_{50} = 1.3 μM), was slightly more potent than compound **2**, whereas compound **7** (EC_{50} = 1.4 ± 0.3, SI = 62.4) showed similar potency and selectivity. In fact, it was actually the most potent compound within the *para* monosubstitued compounds on ring B. The oxidation of the sulfide **10** to sulfoxide (**11**) or sulfone (**12**) led to a reduction in the leishmanicidal activity.

The activity of compounds with a multi-substituted ring B was highly dependent on the nature, number and position of substituents. The activity decreased for an *ortho/para* difluoro substitution (**13**, EC_{50} = 7.1 μM), whereas in the case of the dichloro compounds, **14** (*ortho/para)* and **15 (***meta/para)* preserved the activity of **7**; however, the SI for **15** was better than for **14**.

Table 1. Structure, leishmanicidal activity and cytotoxicity of imidazoisoindolol derivatives.



Compound	R^1	R^2	Intracellular amastigotes EC_{50} (μM)[a]	THP-1 CC_{50} (μM)[b]	MRC-5 CC_{50} (μM)[b]	SI[c]	Promastigotes EC_{50} (μM)[d]	Promastigotes IC_{50} (μM)[e]
1	H	H	27.8 ± 2.9	>200	182.2 ± 2.1	-	nd	nd
2	H	4-Cl	**2.1 ± 0.3**	145.1 ± 3.1	79.9 ± 3.0	**69.1**	11.8 ± 6.8	nd
3	7(8)-Me	H	9.2 ± 0.8	164.6 ± 13.2	125.6 ± 11.9	17.9	nd	nd
4	7(8)-Me	2-Cl	**1.8 ± 0.0**	80.7 ± 8.6	58.4 ± 3.7	**44.8**	**5.9 ± 0.8**	16.7 ± 2.5
5	7(8)-Me	3-NO_2	> 10	182.8 ± 9.6	121.9 ± 5.3	<18.3	nd	nd
6	7(8)-Me	4-F	5.7 ± 0.4	173.2 ± 3.6	111.1 ± 2.9	30.3	nd	nd
7	7(8)-Me	4-Cl	**1.4 ± 0.3**	84.6 ± 2.6	64.4 ± 1.6	**61.5**	**6.1 ± 1.3**	**14.3 ± 1.8**
8	7(8)-Me	4-OH	7.8 ± 2.6	>250	138.9 ± 5.7	>32.2	nd	nd
9	7(8)-Me	4-OMe	> 10	176.8 ± 1.3	120.3 ± 1.9	<17.7	nd	nd
10	7(8)-Me	4-SMe	**1.3 ± 0.0**	80.5 ± 5.8	36.2 ± 3.9	**62.4**	**5.1 ± 1.0**	16.2 ± 1.3
11	7(8)-Me	4-SOMe	>10	>200	>200	-	nd	nd
12	7(8)-Me	4-SO_2Me	>10	>200	181.9 ± 22.3	-	nd	nd
13	7(8)-Me	2,4-diF	7.1 ± 1.1	162. ± 5.3	78.5 ± 3.3	23.0	nd	nd
14	7(8)-Me	2,4-diCl	**1.3 ± 0.2**	22.2 ± 0.0	15.7 ± 0.4	16.7	12.7 ± 3.4	nd
15	7(8)-Me	3,4-diCl	**1.3 ± 0.2**	38.6 ± 0.9	29.9 ± 2.7	**29.3**	10.5 ± 1.8	nd
16	7(8)-Me	3,4-OCH_2O	> 10	>250	121.2 ± 16.1	-	nd	nd
17	7(8)-Me	3,4-diOMe	> 10	246.3 ± 5.3	187.3 ± 33.4	< 24.6	nd	nd
18	7(8)-Me	3,4,5-triOMe	> 10	191.6 ±12.3	113.6 ± 11.9	<19.2	nd	nd
19	7(8)-Me	α-naphthyl	3.1 ± 0.4	42.2 ± 4.1	20.5 ± 1.8	13.5	nd	nd
20	7(8)-Me	β-naphthyl	3.4 ± 0.5	42.4 ± 1.2	20.1 ± 1.8	12.4	nd	nd
21	6(9)-NO_2	4-Cl	>10	146.6 ± 23.0	112.4 ± 11.5	<14.6	nd	nd
22	7(8)-CH_2OH	4-Cl	>10	>200	122.0 ± 18.4	>20	nd	nd
23	7(8)-CHO	4-Cl	8.3 ± 0.8	153.4 ± 11.9	110.2 ± 22.1	18.5	nd	nd
24	7(8)-NO_2	4-Cl	**1.9 ± 0.4**	69.0 ± 8.2	47.2 ± 1.5	**35.6**	**7.1 ± 1.1**	nd
25	6,9-diCl	2-Cl	4.2 ± 0.2	82.0 ± 0.6	41.0 ± 4.0	19.7	nd	nd
26	6,9-diCl	4-Cl	**2.6 ± 0.2**	68.7 ± 2.2	33.8 ± 0.3	26.2	13.3 ± 1.7	**14.4 ± 3.6**
27	6,9-diCl	4-SMe	6.7 ± 0.8	84.4 ± 0.8	77.4 ± 8.2	12.7	nd	nd
28	7,8-diCl	4-Cl	**3.0 ± 0.4**	31.9 ± 5.9	8.9 ± 2.8	10.8	**6.9 ± 1.0**	nd
29	7,8-diCl	4-SMe	**1.0 ± 0.1**	31.1 ± 1.1	8.5 ± 1.5	**31.1**	**2.1 ± 0.4**	**7.3 ± 1.5**
30	6(9)-NO_2	3,4-diCl	**2.5 ± 0.1**	35.0 ± 2.7	31.3 ± 1.8	13.8	9.4 ± 0.9	nd
31	7(8)-NO_2	3,4-diCl	**1.1 ± 0.3**	36.0 ± 2.7	28.8 ± 1.2	**33.3**	**7.6 ± 1.7**	**14.7 ± 2.9**
AmB			**0.1 ± 0.0**	**16.0 ± 2.1**	**12.0 ± 1.5**	**177.3**	**0.1 ± 0.0**	

[a] As specified in Experimental section, activity of the compounds on intracellular amastigotes was measured by the luciferase test; data were expressed as EC_{50} ± SD, half maximal effective concentration. [b] Cytotoxic concentration 50 (CC_{50}) was measured by the inhibition of MTT reduction. [c] Selectivity index (SI) was defined as the ratio: CC_{50} THP-1/EC_{50} for intracellular amastigotes. [d] EC_{50} stands for the concentration of the respective compound that inhibited promastigote proliferation by 50%. To this end, MTT reduction was measured after 72 h of promastigote proliferation in the presence of the respective control. [e] IC_{50} stands for the concentration of the compound that inhibited MTT reduction after 4 h incubation of promastigotes with the compound, hence of its short term effects. Data were expressed as the corresponding parameter ± SD. Three independent experiments were carried out; nd= not determined. Amphotericin B (AmB) was used as reference drug. Values highlighted for comparison purposes, in **red** those of most potent compounds with $EC_{50} \leq 3\mu M$, in **blue** those compounds with EC_{50} values between 3 and 10 μM.

In ring B, neither a methylenedioxy substitution (**16**), nor the presence of two (**17**) or three methoxy groups (**18**), improved the activity respect to compound **9**, with a single methoxy substitution. The presence of an enlarged aromatic group as α–naphthyl, **19 (**EC_{50} = 3.1 μM), and β-naphthyl, **20 (**EC_{50} = 3.4 μM), led to a leishmanicidal activity similar to compound **2**, but with an impaired SI (13.5 and 12.4, respectively *versus* the SI = 69 of compound **2**).

Altogether, we can conclude that the best substitution on ring B (compounds **3** to **20**) to kill *L. donovani* intracellular amastigotes, was the *p*-methylsulfanyl (**10**), followed by the *m*,*p*-dichloro (**15**), and the *p*-chloro (**7**) substitutions. In contrast, the ranking for SI was **10**, **7** and **15**.

Based on the previous results, some deactivating substitutions on ring A, combined with the best ones found on ring B (*p*-Cl, *p*-SMe and *m*,*p*-diCl) were further analysed. Ring A was mono-substituted at positions 6 or 9 and 7 or 8, or di-substituted at positions 6,9 and 7,8. Among the set of compounds with a *p*-chloro on ring B, and monosubstitution at ring A, **21** (6(9)-NO_2), **22** [7(8)-CH_2OH], **23** [7(8)-CHO] and **24** [7(8)-NO_2], compound **24**, with a nitro group at position 7 or 8 showed the best activity (EC_{50} = 1.9 μM, SI = 35.6), rather similar to **4** (with 7(8)-Me on ring A and *o*-chloro in ring B).

To note, compound **24**, with a 7,(8)-nitro substitution, has better leishmanicidal activity than **21** with a 6(9)-nitro substitution. However, the activity of **30** (EC_{50} = 2.5 μM, and SI = 13.8), with the 6(9)-NO_2 and two chlorines (*m*,*p*-diCl) on ring B, was similar to **2**. The 7(8)-nitro derivatives, with one or two chlorine atoms in ring B showed good results (**24**, EC_{50} = 1.9, SI =35.6; **31**, EC_{50} =1.1 μM, SI=33.3). Thus, the addition of one *m*-Cl to a *p*-Cl on ring B improved the leishmanicidal activity. In fact, compound **31** is the second most potent compound of this series.

The best compound, with a 6,9-dichloro substitution on ring A, was **26 (**EC_{50} = 2.6, SI = 26.2), with a *p*-Cl at ring B. Overall, the most potent compound of the series was **29** (EC_{50} = 1.0, SI = 31.1) with 7,8-diCl on ring A and *p*-SMe on ring B.

Additionally, the most potent compounds on intracellular amastigotes (EC_{50} < 3 μM: **2**, **4**, **7**, **10**, **14**, **15**, **24**, **26**, **28**, **29**, **30** and **31**) were assayed on *L. donovani* promastigotes. From these, compound **29** was also the most potent (with EC_{50} = 2.1 μM), followed in decreasing order by compounds **10** (7(8)-Me / *p*-SMe), **4** (7(8)-Me / *o*-Cl) ≅ **7** (7(8)-Me / *p*-Cl), **28** (7,8-diCl / *p*-Cl) ≅ **24** (7(8)-NO_2 / *p*-Cl), **30** (6(9)-NO_2 / *m*,*p*-diCl) and **31** (7(8)-NO_2 / *o*,*p*-diCl). Noteworthy, the IIOLs were more potent against the intracellular *L. donovani* amastigotes than on the promastigote forms of this parasite.

In order to define the primary target of imidazoisoindols, their effects on promastigotes were monitored after a short incubation (t = 4 h) with the selected compounds. Under these experimental conditions, inhibition of MTT reduction, carried out by mitochondrial dehydrogenases, was measured immediately after the end of the incubation, and used as a parameter to select those concentrations of the drug that decreased the viability of parasites. The experimental conditions and parasite model were selected in order to assess the real effects of IIOLs on the energy metabolism of *Leishmania* as their ultimate target: ***i)*** short incubations times minimize the variation of energy-metabolism as a secondary effect of the action on other feasible primary targets; ***ii)*** promastigotes were chosen as their proliferation and metabolic rate are much higher than that of intracellular amastigotes, for which a severe metabolic quiescence was reported [23, 24], making them easier cells to highlight anomalies on energy metabolism; ***iii)*** the promastigote, as extracellular form, has free access to the drugs, without the restrictions imposed to intracellular amastigotes by the macrophage as their host cell. Additionally, a putative modulation of the leishmanicidal effects of the macrophages by IIOLs was avoided. Throughout its life cycle, *Leishmania* mostly relies on the oxidative phosphorylation as the powerhouse for its energy metabolism [24].

As expected, the IC_{50} values corresponding to the inhibition of MTT after 4 h incubation were consistently higher than their respective EC_{50} values, the parameter for inhibition of proliferation (Table 1). Consequently, the leishmanicidal mechanism, or at least its ensuing aftermath, did not reach its final end after the 4 h incubation. As such, IC_{50} values were taken as a reference for the evaluation of the rest of the energetic parameters.

Next, the variation in the intracellular levels of ATP caused by a selected set of compounds was assessed on promastigotes of the *Leishmania* 3-Luc strain. These parasites report ATP variations in living parasites in real time, due to the expression of a cytoplasmic form of firefly luciferase, unable to be imported into the glycosomes, as occurs with the native enzyme. Furthermore, the poor membrane permeability of D-luciferin at pH 7.0 was overcome using a caged luciferin ester (DMNPE-luciferin) [25]. The variation in luminescence were assessed either immediately after the addition of the respective IIOL (Figure 1), or after a 4 h incubation (Figure 2). As expected, the immediate effect of IIOL addition on the inhibition of luminescence (Figure 1) was consistently lower than that measured after 4 h incubation (Figure 2), and their percentages for luminescence inhibition were closer to their respective IC_{50} (Figure 2). In this experiment, IIOL **29** resulted as the most active compound, followed by **26** and **31** for the two incubation time modalities. The decrease in ATP levels was also observed for the other three tested compounds, although for compound **4** the drop in ATP levels after the first 30 min of incubation was practically negligible (Figure 1).

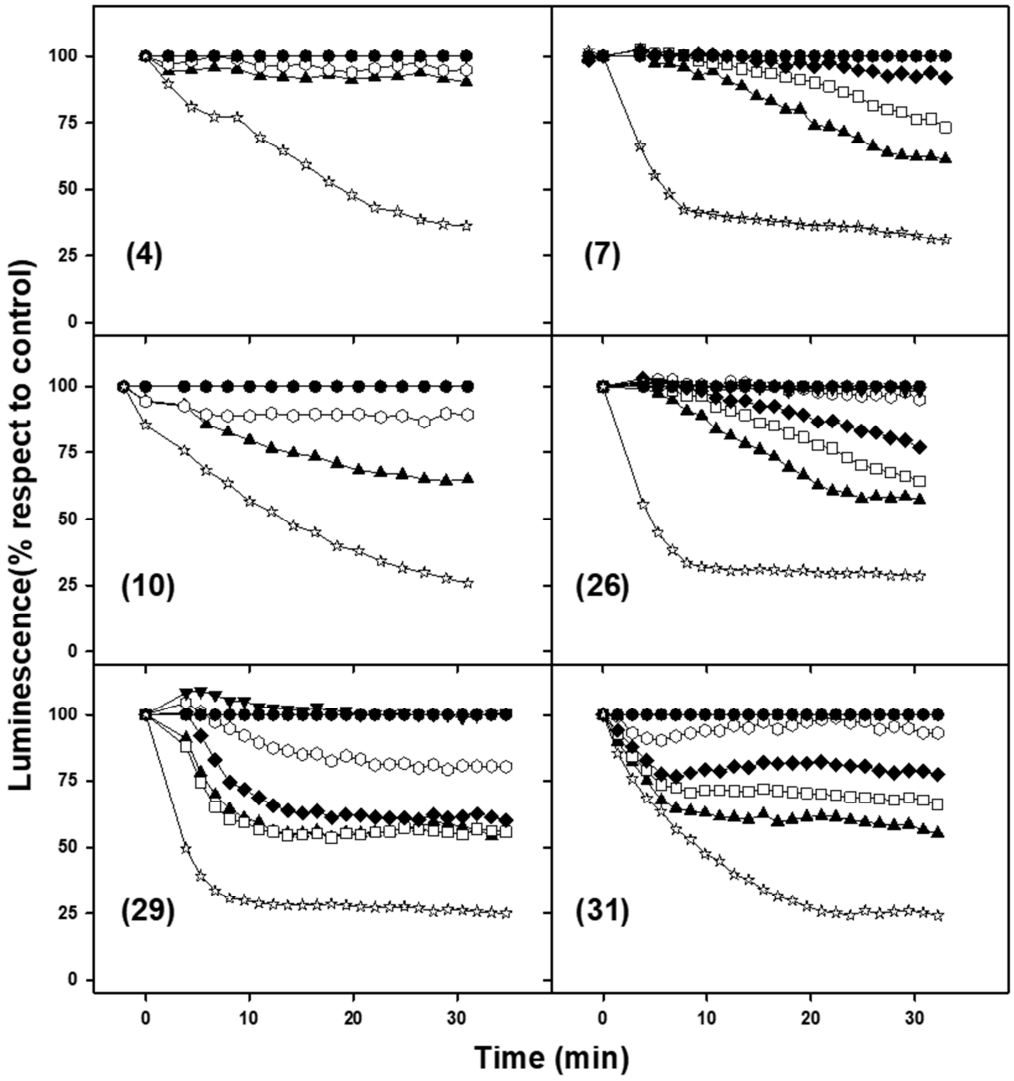


Figure 1. Variation of luminescence of 3-luc *L. donovani* promastigotes after imidazoisoindolols 4, 7, 10, 26, 29 and 31 addition.

Imidazoisoindolols at the respective concentration were added at a promastigotes suspension in RPMI-Øred medium (20×10^6 cells/mL) in the presence of 50 μM DMNPE-D-luciferin. Afterwards, variation of the luminescence of living parasites was monitored in a real time basis. The number of the compound is included inside its corresponding panel. Luminescence was expressed as the percentage respect to untreated parasites. To improve the clarity of the graphic only the significant concentrations for each imidazoisoindolol were represented, although the full set of concentrations (5, 10, 20, 30, 40, and 50 μM) were assayed for each of the selected compounds. Parasites treated with 1.5 μM of 1,4-naphthoquinone were used as internal control. Legend: Imidazoisoindolol concentration (μM): 50 (▲); 40 ( ); 30 (♦); 20 (○); 10 (▼); 1,4-naphthoquinone (1.5 μM, ☆).

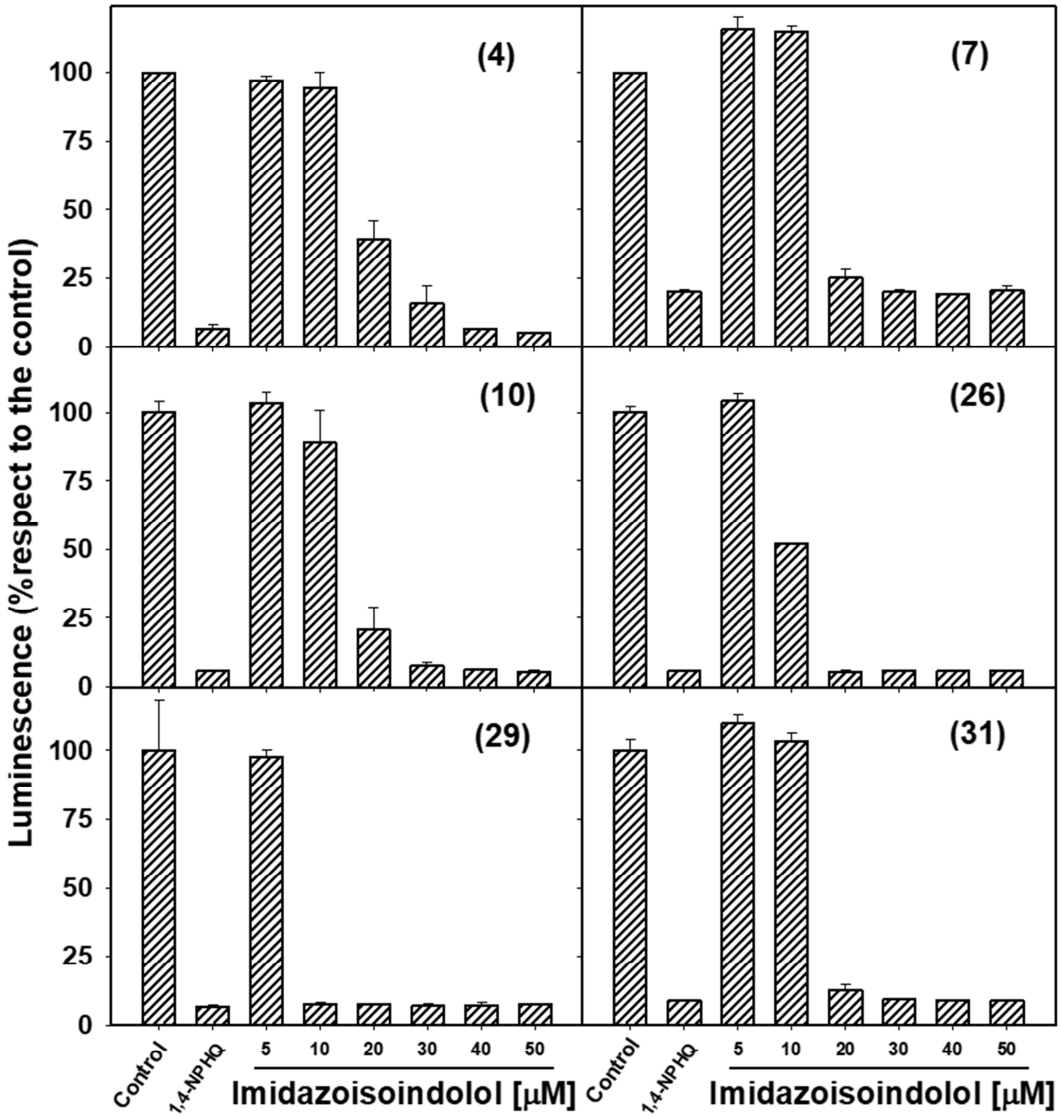


Figure 2. Variation of intracellular ATP levels in *L. donovani* promastigotes after 4h incubation with imidazoisoindolols 4, 7, 10, 26, 29 and 31.

Imidazoisoindolols at the respective concentrations were added at a promastigotes suspension in RPMI-Øred medium (20×10^6 cells/mL), and incubated for 4 h at 26 ºC. Afterwards DMNPE-luciferin was added (50 μM, final concentration) and the luminescence recorded for 10 min. Luminescence values were represented as the percentage respect to untreated controls. Afterwards, variation of the luminescence of living parasites was monitored in a real time basis. The number of the compound is included inside its corresponding panel. Luminescence was expressed as the percentage respect to untreated parasites. Parasites treated with 1.5 μM 1,4-naphthoquinone (1,4-NPHQ) were used as positive control.

In a further step, the origin of the decrease of ATP was approached. In *Leishmania*, the contribution of oxidative phosphorylation to fulfil the global demand of ATP is much higher than glycolysis, especially for intracellular amastigotes [23]. Therefore, to gauge the mitochondrial functionality of

the parasites, the electrochemical potential of mitochondria (ΔΨm), assessed by the intracellular accumulation of Rhodamine 123 (Rh123), is a reliable parameter. This probe has a positive charge delocalized by resonance, behaving as a hydrophobic cation. Consequently, it distributes across both sides of the organelle membranes in response to the membrane potential following the Nernst equation [24]. Rh123 is almost exclusively accumulated inside the mitochondrion, as the organelle with the highest ΔΨm. As can be seen in Figure 3, compound **29** again produced the highest inhibition, leading to a full depolarization at 30 μM, followed by compounds **26** and **31**. The other three compounds assayed, **4**, **7** and **10**, showed a decrease in Rh123 accumulation although at concentrations considerable higher than the aforementioned compounds.

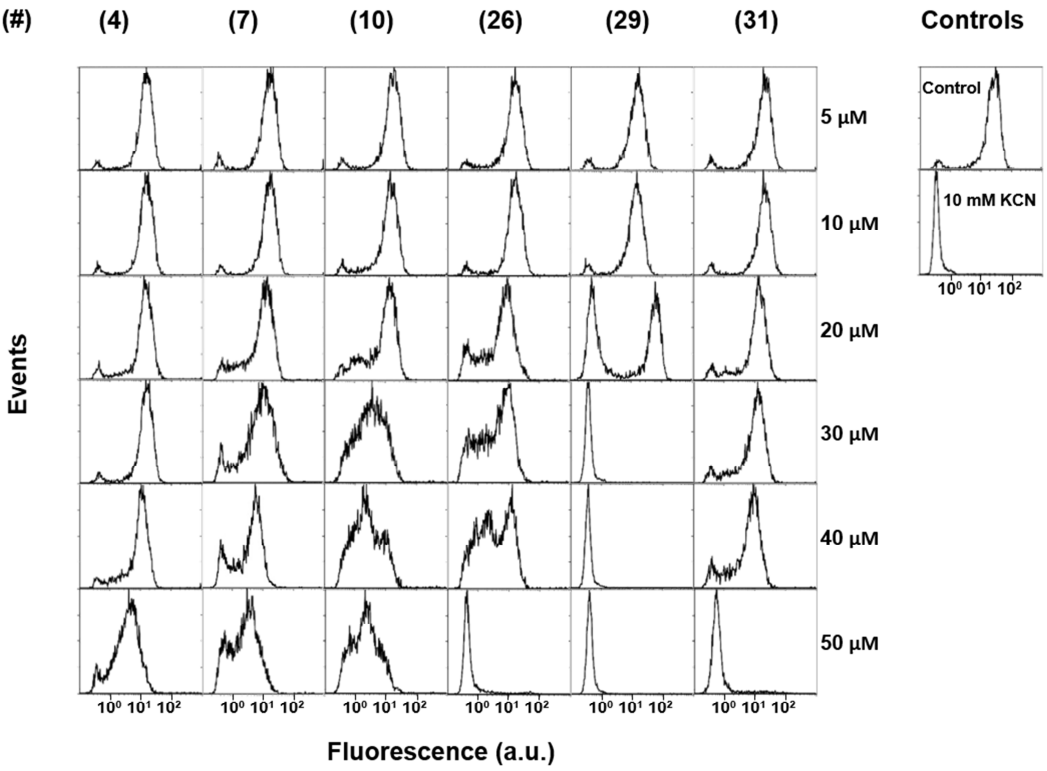


Figure 3. Rhodamine 123 incorporation to *L. donovani* promastigotes after incubation with imidazoisoindolols 4, 7, 10, 26, 29 and 31.

Promastigotes were resuspended at 20 × 10^6 cells/mL in RPMI-Øred and incubated with the corresponding concentration of imidazoisoindolols for 4 h. Afterwards, parasites were incubated with Rh123, and the intracellular accumulation of the probe measured by flow cytometry (λ_{exc}= 488nm, λ_{em} = 520 nm). Parasites incubated with 10 mM KCN were used as a control for a dysfunctional mitochondrion.

The induction of membrane permeability is the other most likely alternative to account for the loss of ATP. Under a permeabilization process, the ionic gradients across the plasma membrane were dissipated, leading to a futile hydrolysis of ATP by the ionic gradients; additionally, the import of metabolites carried out by electrochemical transporters is jeopardized. If the membrane lesion is severe enough, cytoplasmic content, including nucleotides may leak [25]. Plasma membrane permeabilization was monitored by the increase in fluorescence of SYTOX green, a membrane

impermeable intercalating agent. For compounds **26**, **29**, and **31**, a concentration dependent increase of SYTOX green fluorescence was observed. The most pronounced increase was for compound **29**, evident at 10 μM, whereas compound **31** showed the slowest permeabilization rate (Figure 4).

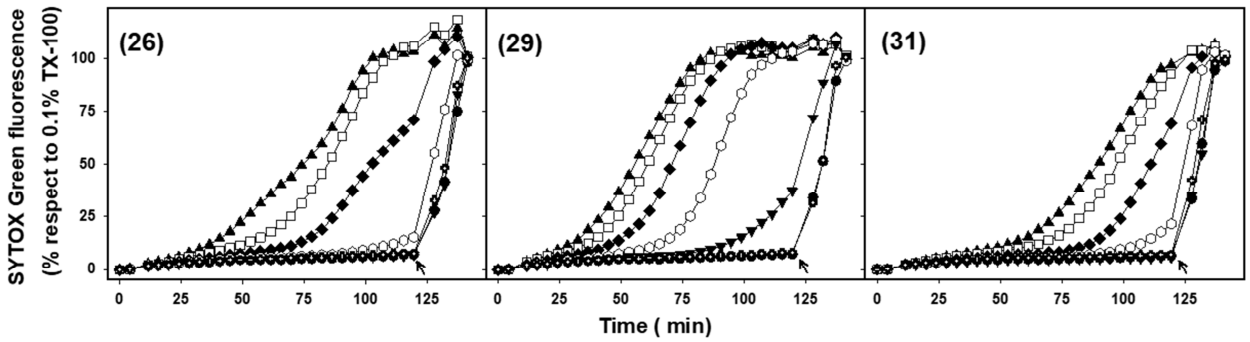


Figure 4. Entrance of the vital dye SYTOX green in *L. donovani* promastigotes induced by imidazoisoindolols 26, 29 and 31.

Imidazoisoindolols at their respective concentration was added to a promastigotes suspension (20 x 10^6 cells/mL) resuspended in RPMI-Øred containing 1.0 μM SYTOX green, and the increase in fluorescence monitored (ʎ = 485 nm; $ʎ_{em}$= 520 nm). Triton X-100 (0.1%, final concentration) was added at the point indicated by an arrow, and this fluorescence considered as 100%. Only the traces of the significant IIOL concentrations were included in each panel to avoid confusion due to overlapping traces. Legend: IIOL concentration (μM): 50 (▲); 40 ( ); 30 (♦); 20 (○); 10 (▼).

For concentrations close to their IC_{50}, the increase in fluorescence for these three compounds remained unnoticed at least 20 min after their addition to the parasites, even for the highest concentration assayed (50 μM) (Figure 4). Even for compound **29** at 10 μM, this lag was close to 1h after addition. By electron microscopy some blebbings of the plasma membrane were visualized in promastigotes treated overnight with compound **29** at 10 μM (~ 70% inhibition of MTT reduction) (Figure 5). Nevertheless, this damage to the plasma membrane is far less severe than that produced by typical membrane agents, as some antimicrobial peptides, with massive release of cytoplasmic material [26]. The micrograph of *L. donovani* promastigote treated with IIOL **29** (Figure 5, panel B) showed swelling of the mitochondrial cristae suggesting at least a partial independence of the mitochondrial damage with a massive membrane permeabilization not observed in the same micrograph. This hypothesis agrees with the experimental kinetics of the decrease of ATP levels and entrance of SYTOX green. The intracellular accumulation of SYTOX green is a process close to irreversibility, due to the high affinity of this probe for DNA. Hence, even transitory and reversible lesions may lead with time to a significant intracellular concentration of the dye in the absence of severe membrane permeabilization.

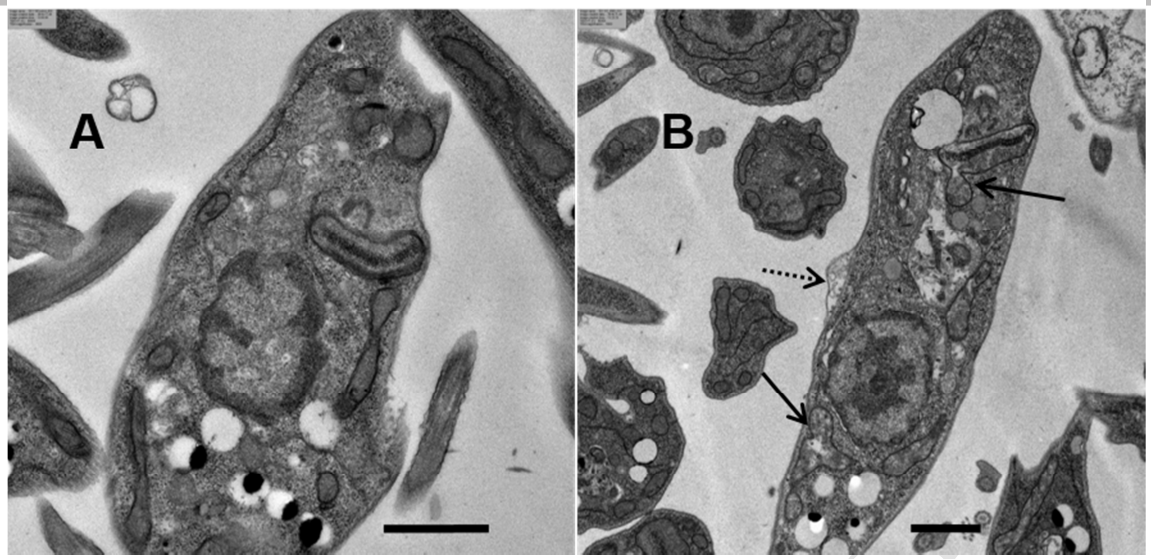


Figure 5. Transmission electron micrographs of *L. donovani* promastigotes treated with imidazoisoindolol 29.

Parasites were incubated with 10 μM imidazoisoindolol **29** for 12 h in complete growth medium. **Panel A**.- Control parasites. **Panel B**.- Parasites treated with 10 μM of **29**. Solid arrows pinpoint damaged mitochondrial areas. Dashed arrow points out to a blebbing in the plasma membrane of the parasite. Magnification bar = 1μm.

In this regard, a low permeability status of the plasma membrane requires the maintenance of a phospholipid asymmetry, for which flippases and floppases, both dependent on ATP hydrolysis, played a major role [27], thus a mild damage to the plasma membrane after a severe ATP depletion cannot be ruled out. In all, the experimental results support that the plasma membrane permeabilization is rather an effect than the origin of the leishmanicidal activity of imidazoisoindolols.

The single mitochondrion of trypanosomatids is an appealing chemotherapeutical target, as demonstrated by the ample spectrum of chemical structures successfully assayed for leishmanicidal activity with mitochondrial involvement [28-30]. To note, two of the drugs under current clinical use, paromomycin and miltefosine, have the mitochondrion of *Leishmania* as one of their respective targets [31, 32]. In *Leishmania* as in other trypanosomatids, there is only a single mitochondrion per cell [33]; this may ruled out a hypothetical functional compensation by other unharmed mitochondria [30]. Additionally, a deficit in the mitochondrial contribution to the energy metabolism of the parasites, can only partially offset by an increase in the glycolytic capacity [34]. The infection of the macrophages by *Leishmania* shifts the energy metabolism of the host cells from a glycolytic pattern into a more relevant contribution oxidative phosphorylation [35]. Even when some of the best IIOLs tested in this work have SI > 30, we cannot discard that the susceptibility of *Leishmania* infected macrophages will be higher than the non-infected ones, due to their higher dependence on oxidative phosphorylation. In this context, under the imidazoisoindolol challenge, we may surmise a metabolic rewiring of the infected macrophages towards glycolysis, supposedly

less favourable for survival and replication of intracellular survival. If this is the case, IIOLs will not be exclusively targeting the parasite, but the host-parasite interphase as well.

In any case, we cannot rule out the existence of other targets for imidazoisoindolols aside the mitochondrial energy metabolism of *Leishmania*, as exemplified with compound **4**.

Most potent compounds were selected for the determination of their physicochemical, ADME, and drug-like properties. These were obtained from OSIRIS [36] and preADMET [37] algorithms accessed online. Thus, clogP values ranged from 2.37 to 4.57; logS values from -3.20 to -4.33 (at pH= 7.5 and 25 ºC); human intestinal absorption (HIA) values resulted higher than 95 % and *in vitro* plasma protein binding (PPB) values under 90%. In addition, all the selected compounds accomplished the Lipinski's Rule of Five [38], and were within the limits established in Comprehensive Medicinal Chemistry Database (CMC-like rule) [39] and the World Drug Index (WDI-like rule) [40], to be considered as adequate drug-like molecules. Finally, they attained an OSIRIS global drug-score qualification in the range 0.52 to 0.79. Even with this favourable profile, it must keep in mind that Lipinsky rules are frequently violated for antiparasiticidal drugs, including miltefosine and amphotericin B, two front-line antileishmanial drugs [41].

3. **Conclusions**

In summary, we have identified several derivatives of imidazo[2,1-*a*]isoindol-5-ol, that exhibit promising leishmanicidal activity. Of the compounds tested, the *para*-monosubstituted 6-benzylimidazo[2,1-*a*]isoindol-5-ols containing chloro (Cl) or methylsulfanyl (MeS) groups attached to the benzyl fragment were the best, with EC_{50} values for intracellular amastigotes in the low micromolar range of concentrations, and SI values higher than 30. Furthermore, the involvement of mitochondrion as a target, at least for the most active one (**29**) was demonstrated, even when additional targets were also suspected.

All these findings will contribute to establishing structure activity relationships to guide further design of new compounds, which may include motifs to promote mitochondrial accumulation, mostly the presence of lipophilic cations, already used for other leishmanicidal molecules, to promote a selective accumulation in this organelle [25]. The message to be conveyed is the proof-of-concept for the inclusion of the IIOL scaffold inside the portfolio of new candidates for preclinical assays against *Leishmania*.

4. **Experimental**

4.1. *Chemistry*

All commercial chemicals (Aldrich, Alpha, Fischer, SDS) were used as purchased and solvents (Fischer, SDS, Scharlau) purified by the standard procedures prior to use [42]. Reactions were monitored by Thin Layer Chromatography (TLC) (Kieselgel 60 F254 precoated plates, E. Merck, Germany), the spots were detected by exposure to UV lamp at λ 254 nm, and colorization with 10% phosphomolybdic acid or ninhydrin spray, and further heating of the plate. Melting points (Mp) were determined with a Büchi apparatus in open capillaries and were uncorrected. Separations by flash column chromatography were performed on neutral alumina Merck 60 silica gel (0.063-0.2 mesh). Infrared spectra were recorded on a FT-IR spectrometer Perkin Elmer, System BX using neat samples, without solvent, or KBr disks. NMR spectra were recorded either on a Bruker ARX-400 (400 MHz for ^{1}H, 100 MHz for ^{13}C) or a Bruker AC 200 MHz (200 MHz for ^{1}H, 50 MHz for ^{13}C). The spectra were measured either in $CDCl_3$, C_6D_6 or DMSO-d_6, using tetramethylsilane (TMS) as internal standard, chemical shifts (δ) are given in ppm and coupling constants (*J*) in Hertz. High resolution mass spectra (HRMS) were obtained by electron spray ionisation-mass spectrometry (ESI-MS) technique (5 kV) on a QSTAR XL mass spectrometer.

4.1.1. *General procedure for the synthesis of intermediate benzal(naphthylmethylidene)phthalides* (BPs, NPs)

2.2 mmol of phthalic anhydride or derivative, 2.7 mmol of the corresponding phenyl/ naphthylacetic acid and 0.26 mmol of sodium acetate with 5 mL of toluene were placed into in a round bottom flask plugged to a Dean-Stark apparatus. The mixture was maintained under N_2 atmosphere at 210-245 ºC, with magnetic stirring, for 9-33 h. After cooling, the reaction mixture was extracted with ethyl acetate and washed successively with (sat) Na_2CO_3, brine and water, dried over Na_2SO_4 and evaporated under reduced pressure to give the crude product. Solid products were purified by crystallization, whereas oils were subjected to flash chromatography on silica gel. The ratio between the 6(7)-regioisomers was determined by comparison of the integral of their respective olefinic protons (signal H-8), in ^{1}H-NMR. All phthalides were obtained as *Z* isomers. Their configuration was confirmed through NOE or ROESY experiments, and final yields ranged between 40 - 95%.

We report here the data of benzalphthalides **BP-1**, **BP-2** and **BP-3**, as precursors to the three most representative IIOLs **26**, **29** and **31**, respectively.

4.1.1.1. *(Z)-4,7-Dichloro-3-(4-chlorobenzylidene)isobenzofuran-1(3*H*)-one*, **BP-1**

Reaction time 16 h. Compound crystallized from MeOH/hexane to give a pale yellow solid, Mp: 207-209 ºC, yield 46%. IR (KBr): ν_{max} 3076, 1774, 1647, 1465, 1224, 1152, 996, 903, 819 cm^{-1}. ^{1}H

NMR (400 MHz, C_6D_6): δ 6.50 (d, *J* = 8.4 Hz, 1H), 6.62 (d, *J* = 8.4 Hz, 1H), 6.87 (s, 1H), 7.15 (d, *J* = 8.6 Hz, 2H), 7.51 (d, *J* = 8.6 Hz, 2H). ^{13}C NMR (100 MHz, C_6D_6): δ 111.1, 122.4, 125.7, 129.0 (2C), 130.7, 131.6, 131.8 (2C), 131.9, 134.8, 135.6, 137.1, 141.8, 161.2. HRMS (ESI^+) calcd. for $C_{15}H_7Cl_3O_2$ $[M+Na]^+$: 346.9404, found. 346.9408.

4.1.1.2. *(Z)-5,6-Dichloro-3-(4-methylsulfanylbenzylidene)isobenzofuran-1(3*H*)-one,* **BP-2**

Reaction time 12 h. Compound crystallized from MeOH/hexane to give a yellow solid, Mp: 200-202 °C. yield: 59%, IR (KBr): ν_{max} 2907, 1757, 1659, 1589, 1312, 1284, 1153, 1082, 929, 888, 816 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.52 (s, 3H), 6.36 (s, 1H), 7.25 (d, *J* = 8.6 Hz, 2H), 7.73 (d, *J* = 8.6 Hz, 2H), 7.85 (s, 1H), 8.00 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 15.3, 108.6, 121.6, 122.6, 125.3, 126.1 (2C), 127.2, 130.6 (2C), 134.0, 139.4, 139.7, 139.9, 141.2, 165.1 HRMS (ESI^+) calcd. for $C_{16}H_{11}Cl_2O_2S$ $[M+H]^+$: 336.9851, found. 336.9840.

4.1.1.3. *(Z)-3-(3,4-Dichlorobenzylidene)-5(6)-nitroisobenzofuran-1(3*H*)-one,* **BP-3**

Reaction time 14 h. Compound crystallized from MeOH/hexane to give an orange solid, Mp: 190-194 ºC, yield: 38%. IR (KBr): ν_{max} 2921, 1778, 1616, 1536, 1344, 1100, 1001, 855, 763 cm^{-1}. HRMS (ESI^+) calcd. for $C_{15}H_8Cl_2NO_4$ $[M+H]^+$: 335.9830, found 335.9839.

*(Z)-3-(3,4-Dichlorobenzylidene)-5-nitroisobenzofuran-1(3*H*)-one,* **BP-3a**. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.26 (s, 1H), 7.71 (dd, *J* = 8.8, 1.2 Hz, 1H), 7.72 (d, *J* = 8.8 Hz, 1H), 7.91 (d, *J* = 1.2 Hz, 1H), 8.17 (d, *J* = 8.4 Hz, 1H), 8.66 (dd, *J* = 8.4, 2.0 Hz, 1H), 8.93 (d, *J* = 2.0 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 106.5, 120.8, 122.5, 127.0, 129.9, 130.0, 131.1 (2C), 131.4, 131.6, 133.5, 144.2 (2C), 148.6, 164.4.

*(Z)-3-(3,4-Dichlorobenzylidene)-6-nitroisobenzofuran-1(3*H*)-one,* **BP-3b**. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.19 (s, 1H), 7.71 (d, *J* = 8.8 Hz, 1H), 7.73 (dd, *J* = 8.8, 1.2 Hz, 1H), 7.95 (d, *J* = 1.2 Hz, 1H), 8.26 (d, *J* = 8.4 Hz, 1H), 8.37 (dd, *J* = 8.4, 2.0 Hz, 1H), 8.60 (d, *J* = 2.0 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 108.1, 116.6, 123.8, 125.2, 127.1, 129.8, 129.9, 130.3, 131.2, 131.6, 133.3, 140.7, 144.1, 151.8, 164.2.

4.1.2. General procedure for the preparation of 5-benzyl(naphthylmethyl)imidazo[*2,1-*a*]isoindol-5-ol derivatives* (compounds **1 - 32)**

1.0 mmol of the corresponding benzyl(*naphthylmethylidene*)phthalide was dissolved into ethylenediamine (4 mL) and heated at 70 – 80 ºC for 7 – 12 h with magnetic stirring. After cooling, the reaction mixture was extracted with ethyl acetate and washed with water. The organic layer was

dried over Na_2SO_4 and concentrated under reduced pressure to give the crude product, which was further purified by column chromatography on silica gel. Reaction yields ranged within 80 - 100%. Compounds **3** to **20**, **22** to **24** and **31** were obtained as mixtures of 7(8)-regioisomers, and compounds **21** and **30** as mixtures of 6(9)-regioisomers, in relation 1:(0.9-0.6) approximately.

*4.1.2.1. 5-Benzyl-3,5-dihydro-2*H*-imidazo[2,1-*a*]isoindol-5-ol,* **1**.

The compound was prepared and characterized as previously described in [23]. Yield: 91%.

*4.1.2.2. 5-(4-Chlorobenzyl)-3,5-dihydro-2*H*-imidazo[2,1-*a*]isoindol-5-ol,* **2**.

The compound was prepared and characterized as previously described in [23]. Yield: 93%.

*4.1.2.3. 5-Benzyl-7(8)-methyl-3,5-dihydro-2*H*-imidazo[2,1-*a*]isoindol-5-ol,* **3**.

Yield: 85%. Oil. IR (NaCl): ν_{max} 3359, 3294, 2925, 1684, 1621, 1393, 1083, 703 cm^{-1}. HRMS (ESI^+) calcd. for $C_{18}H_{19}N_2O$ $[M+H]^+$: 279.1492, found 279.1504.

*5-Benzyl-7-methyl-3,5-dihydro-2*H*-imidazo[2,1-*a*]isoindol-5-ol,* **3a**. Differential assignment of NMR data for pairs of regioisomers based on homo- and heteronuclear 2D-NMR correlations. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.35 (s, 3H), 2.77-3.10 (m, 2H), 3.12 (d, *J* = 14.0 Hz, 1H), 3.41 (d, *J* = 14.0 Hz, 1H), 3.67 (bs, 1H, D_2O), 3.95-4.05 (dt, *J* = 14.5, 2.6 Hz, 2H), 6.77-7.03 (m, 5H), 7.09 (d, *J* = 7.9 Hz, 1H), 7.20 (s, 1H), 7.37 (d, *J* = 7.9 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 22.0, 39.4, 41.8, 44.6, 89.2, 122.9, 126.8, 127.9 (3C), 129.8, 130.0 (2C), 130.9, 135.2, 142.6, 148.3, 167.8.

*5-Benzyl-8-methyl-3,5-dihydro-2*H*-imidazo[2,1-*a*]isoindol-5-ol,* **3b**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.29 (s, 3H), 2.77-3.10 (m, 2H), 3.11 (d, *J* = 14.0 Hz, 1H), 3.22 (d, *J* = 14.0 Hz, 1H), 3.67 (bs, 1H, D_2O), 3.95-4.05 (dt, *J* = 14.5, 2.6 Hz, 2H), 6.77-7.03 (m, 5H), 7.12 (d, *J* = 7.5 Hz, 1H), 7.19 (d, *J* = 7.5 Hz, 1H), 7.30 (s, 1H).^{13}C NMR (50 MHz, $CDCl_3$): δ 21.4, 39.4, 41.8, 44.6, 89.3, 122.2, 123.3, 127.9 (3C), 130.0 (2C), 130.9, 132.8, 138.9, 135.2, 145.2, 167.8.

*4.1.2.4. 5-(2-Chlorobenzyl)-7(8)-methyl-3,5-dihydro-2*H*-imidazo[2,1-*a*]isoindol-5-ol,* **4**.

Yield: 78%. Oil. IR (NaCl): ν_{max} 3292, 2925, 1681, 1620, 1442, 1392, 1080, 1053, 755, 732 cm^{-1}. HRMS (ESI^+) calcd. for $C_{18}H_{18}ClN_2O$ $[M+H]^+$: 313.1110, found 313.1105.

*5-(2-Chlorobenzyl)-7-methyl-3,5-dihydro-2*H*-imidazo[2,1-*a*]isoindol-5-ol,* **4a**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.32 (s, 3H), 2.82-3.15 (m, 2H), 3.32 (d, *J* = 14.0 Hz, 1H), 3.54 (d, *J* = 14.0 Hz, 1H), 4.02-4.12 (dt, *J* = 14.5, 2.2 Hz, 2H), 6.90 (m, 3H), 7.11 (d, *J* = 7.9 Hz, 1H), 7.12 (m, 1H), 7.17 (bs, 1H), 7.45 (d, *J* = 7.9 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 19.3, 36.8, 37.9, 39.1, 86.3, 120.0, 120.4, 123.7, 125.3, 125.5, 126.7, 127.1, 128.9, 130.6, 132.2, 139.9, 145.3, 165.0.

*5-(2-Chlorobenzyl)-8-methyl-3,5-dihydro-2*H-*imidazo[2,1-*a*]isoindol-5-ol,* **4b**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.31 (s, 3H), 2.82-3.15 (m, 2H), 3.32 (d, *J* = 14.0 Hz, 1H), 3.54 (d, *J* = 14.0 Hz, 1H), 4.02-4.12 (dt, *J* = 14.5, 2.2 Hz, 2H), 6.90 (m, 3H), 7.11 (m, 1H), 7.12 (d, *J* = 8.0 Hz, 1H), 7.18 (d, *J* = 8.0 Hz, 1H), 7.38 (*s*, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 18.7, 36.8, 37.9, 39.1, 86.3, 119.7, 120.6, 123.7, 125.3, 125.5, 126.7, 128.9, 130.0, 130.6, 132.2, 143.0, 145.0, 165.0.

*4.1.2.5. 7(8)-Methyl-5-(3-nitrobenzyl)-3,5-dihydro-2*H-*imidazo*[*2,1-*a]*isoindol-5-ol,* **5**.

Yield: 68%. Oil. IR (NaCl): ν_{max} 3354, 3294, 2923, 1684, 1528, 1350, 1338, 1085, 824 cm^{-1}. HRMS (ESI$^+$) calcd. for $C_{18}H_{18}N_3O_3$ $[M+H]^+$: 324.1343, found 324.1338.

*7-Methyl-5-(3-nitrobenzyl)-3,5-dihydro-2*H-*imidazo*[*2,1-*a]*isoindol-5-ol,* **5a**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.31 (s, 3H), 3.10 (d, *J* = 14.0 Hz, 1H), 3.35 (d, *J* = 14.0 Hz, 1H), 3.72 (m, 2H), 3.79 (m, 2H), 6.96 (m, 3H), 7.22 (m, 1H), 7.24 (d, *J* = 8.0 Hz, 1H), 7.61 (dd, *J* = 8.0, 7.8 Hz, 1H), 7.79 (d, *J* = 8.0 Hz, 1H), 7.80 (m, 1H), 8.09 (m, 1H), 8.14 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 22.0, 39.2, 41.8, 44.2, 88.8, 121.9, 122.0, 122.8, 125.1, 128.1, 128.8, 130.2, 136.0, 139.5, 143.1, 147.7, 147.8, 167.6.

*8-Methyl-5-(3-nitrobenzyl)-3,5-dihydro-2*H-*imidazo*[*2,1-*a]*isoindol-5-ol,* **5b**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.28 (s, 3H), 3.10 (d, *J* = 14.0 Hz, 1H), 3.35 (d, *J* = 14.0 Hz, 1H), 3.60 (bs, 1H, D_2O), 3.72 (m, 2H), 3.79 (m, 2H), 6.96 (m, 3H), 7.30 (d, *J* = 8.1 Hz, 1H), 7.34 (d, *J* = 8.1 Hz, 1H), 7.61 (dd, *J* = 8.0, 7.8 Hz, 1H), 7.77 (bs, 1H), 7.80 (m, 1H), 8.09 (m, 1H), 8.14 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.4, 39.2, 41.8, 44.2, 88.8, 121.9 (2C), 123.0, 125.1, 128.8, 130.8, 133.1, 136.0, 137.3, 139.5, 144.5, 147.5, 167.6.

*4.1.2.6. 5-(4-Fluorobenzyl)-7(8)-methyl-3,5-dihydro-2*H-*imidazo*[*2,1-*a]*isoindol-5-ol,* ***6***.

Yield: 98%. Oil. IR (NaCl): ν_{max} 3352, 3293, 2926, 1683, 1620, 1511, 1223, 1077, 1053, 832, 781, 726 cm^{-1}. HRMS (ESI$^+$) calcd. for $C_{18}H_{18}FN_2O$ $[M+H]^+$: 297.1397, found 297.1381.

*5-(4-Fluorobenzyl)-7-methyl-3,5-dihydro-2*H-*imidazo*[*2,1-*a]*isoindol-5-ol,* **6a**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.42 (s, 3H), 2.36-3.09 (m, 2H), 3.28 (d, *J* = 14.0 Hz, 1H), 3.39 (d, *J* = 14.0 Hz, 1H), 3.63 (bs, 1H, D_2O), 3.90-4.14 (m, 2H), 6.72-6.82 (m, 4H), 7.32 (bs, 1H), 7.33 (m, 1H), 7.46 (d, *J* = 7.5 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 22.0, 39.2, 41.8, 43.8, 89.0, 114.9 (d, *J* = 7.4 Hz, 2C), 122.9, 123.3, 128.2, 130.9, 131.3, 131.5 (d, *J* = 28.1 Hz, 2C), 142.7, 148.2, 162.4 (d, *J* = 244 Hz), 167.7.

*5-(4-Fluorobenzyl)-8-methyl-3,5-dihydro-2*H-*imidazo*[*2,1-*a]*isoindol-5-ol,* **6b**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.37 (s, 3H), 3.09-2.36 (m, 2H), 3.28 (d, *J* = 14.0 Hz, 1H), 3.39 (d, *J* = 14.0 Hz, 1H), 3.63 (s, 1H, D_2O), 4.14-3.90 (m, 2H), 6.82-6.72 (m, 4H), 7.18 (d, *J* = 8.0 Hz, 1H), 7.30 (m, 1H), 7.39 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.4, 39.2, 41.8, 43.8, 89.0, 114.9 (d, *J* = 7.4 Hz, 2C),

122.0, 122.8, 128.2, 130.9, 131.5 (d, *J* = 28.1 Hz, 2C), 132.8, 139.0, 145.0, 162.4 (d, *J* = 244 Hz), 167.7.

*4.1.2.7. 5-(4-Chlorobenzyl)-7(8)-methyl-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **7**.

Yield: 86%. Oil. IR (NaCl): ν_{max} 3258, 2925, 1679, 1620, 1490, 1115, 1080, 815, 728 cm^{-1}. HRMS (ESI^+) calcd. for $C_{18}H_{18}ClN_2O$ $[M+H]^+$: 313.1110, found 313.1106.

*5-(4-Chlorobenzyl)-7-methyl-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **7a**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.37 (s, 3H), 2.74-3.36 (m, 2H), 3.10 (d, *J* = 14.0 Hz, 1H), 3.33 (d, *J* = 14.0 Hz, 1H), 3.81 (bs, 1H, D_2O), 3.96-4.09 (m, 2H), 6.72 (d, *J* = 8.3 Hz, 2H), 7.00 (d, *J* = 8.3 Hz, 2H), 7.14 (s, 1H), 7.16 (d, *J* = 7.9 Hz, 1H), 7.41 (d, *J* = 7.9 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.9, 39.3, 41.7, 43.8, 89.0, 122.7, 128.0 (3C), 129.8, 130.8, 131.2 (2C), 132.7, 133.6, 142.7, 148.0, 167.6.

*5-(4-Chlorobenzyl)-8-methyl-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **7b**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.32 (s, 3H), 2.74-3.36 (m, 2H), 3.10 (d, *J* = 14.0 Hz, 1H), 3.33 (d, *J* = 14.0 Hz, 1H), 3.81 (bs, 1H, D_2O), 3.96-4.09 (m, 2H), 6.72 (d, *J* = 8.3 Hz, 2H), 7.00 (d, *J* = 8.3 Hz, 2H), 7.12 (d, *J* = 8.8 Hz, 1H), 7.25 (d, *J* = 8.8 Hz, 1H), 7.32 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.3, 39.3, 41.7, 43.8, 88.9, 122.8, 123.3, 128.0 (2C), 130.8, 131.2 (2C), 132.5, 132.7, 133.6, 139.0, 144.8, 167.6.

*4.1.2.8. 5-(4-Hydroxybenzyl)-7(8)-methyl-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **8**.

Yield: 53%. Oil. IR (NaCl): ν_{max} 3354, 2921, 1668, 1616, 1440, 1231, 1072, 831 cm^{-1}. HRMS (ESI^+) calcd. for $C_{18}H_{19}N_2O_2$ $[M+H]^+$: 295.1441, found 295.1442.

*5-(4-Hydroxybenzyl)-7-methyl-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **8a**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.35 (s, 3H), 2.79-2.96 (m, 2H), 3.25 (d, *J* = 14.0 Hz, 1H), 3.40 (d, *J* = 14.0 Hz, 1H), 3.79-3.88 (m, 2H), 6.43 (d, *J* = 8.3 Hz, 2H), 6.54 (d, *J* = 8.3 Hz, 2H), 7.17 (d, *J* = 1.1, 1H), 7.24 (dd, *J* = 7.9, 1.1 1H), 7.34 (d, *J* = 7.9, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.9, 39.9, 41.3, 42.6, 90.6, 114.8 (2C), 122.9, 123.0, 125.4, 128.1, 130.0, 130.8 (2C), 143.0, 147.6, 155.5, 168.5.

*5-(4-Hydroxybenzyl)-8-methyl-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **8b**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.28 (s, 3H), 2.79-2.96 (m, 2H), 3.25 (d, *J* = 14.0 Hz, 1H), 3.40 (d, *J* = 14.0 Hz, 1H), 3.79-3.88 (m, 2H), 6.43 (d, *J* = 8.3 Hz, 2H), 6.53 (d, *J* = 8.3 Hz, 2H), 7.10 (d, *J* = 7.9 Hz, 1H), 7.20 (d, *J* = 7.9, 1H), 7.26 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.2, 39.9, 41.3, 42.6, 90.6, 114.8 (2C), 122.3, 122.6, 125.4, 128.1, 130.8 (2C), 133.0, 139.3, 144.5, 155.5, 168.5.

*4.1.2.9. 5-(4-Methoxybenzyl)-7(8)-methyl-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **9**.

Yield: 94%. Oil. IR (NaCl): ν_{max} 3357, 2929, 1680, 1612, 1513, 1249, 1035, 819, 756 cm^{-1}. HRMS (ESI^+) calcd. for $C_{19}H_{21}N_2O_2$ $[M+H]^+$: 309.1598, found 309.1609.

*5-(4-Methoxybenzyl)-7-methyl-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **9a**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.42 (s, 3H), 2.71-2.93 (m, 2H), 3.22 (d, *J* = 13.9 Hz, 1H), 3.36 (d, *J* = 13.9 Hz, 1H), 3.57 (bs, 1H, D_2O), 3.68 (s, 3H), 3.98-4.08 (m, 2H), 6.60 (d, *J* = 8.8 Hz, 2H), 6.65 (d, *J* = 8.8 Hz, 2H), 7.18 (bs, 1H), 7.28 (d, *J* = 7.7 Hz, 1H), 7.44 (d, *J* = 7.7 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.9, 39.5, 41.7, 43.2, 55.0, 89.6, 113.2 (2C), 122.6, 122.8, 128.2, 129.7, 130.4, 130.8 (2C), 148.1, 142.5, 158.2, 167.9.

*5-(4-Methoxybenzyl)-8-methyl-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **9b**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.36 (s, 3H), 3.00-3.20 (m, 2H), 3.23 (d, *J* = 13.9 Hz, 1H), 3.36 (d, *J* = 13.9 Hz, 1H), 3.57 (bs, 1H, D_2O), 3.68 (s, 3H), 3.98-4.08 (m, 2H), 6.60 (d, *J* = 8.8 Hz, 2H), 6.65 (d, *J* = 8.8 Hz, 2H), 7,16 (d, *J* = 7.7 Hz, 1H), 7.23 (d, *J* = 7.7 Hz, 1H), 7.36 (bs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.3, 39.5, 41.7, 43.2, 55.0, 89.5, 113.2 (2C), 122.1, 123.1, 127.0, 128.2, 130.9 (2C), 132.6, 138.8, 144.9, 158.2, 167.8.

*4.1.2.10. 7(8)-Methyl-5-(4-methylsulfanylbenzyl)-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **10**. Yield: 94%. Oil. IR (NaCl): ν_{max} 3294, 2922, 1682, 1620, 1405, 1080, 819, 732, 703 cm^{-1}. HRMS (ESI^+) calcd. for $C_{19}H_{21}N_2OS$ $[M+H]^+$: 325.1369, found 325.1376.

*7-Methyl-5-(4-methylsulfanylbenzyl)-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **10a**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.34 (s, 3H), 2.38 (s, 3H), 2.98-3.21 (m, 2H), 3.11 (d, *J* = 14.0 Hz, 1H), 3.33 (d, *J* = 14.0 Hz, 1H), 3.95-4.15 (m, 2H), 4.20 (bs, 1H, D_2O), 6.73 (d, *J* = 8.2 Hz, 2H), 6.93 (d, *J* = 8.2 Hz, 2H), 7.19 (bs, 1H), 7.26 (d, *J* = 7.5 Hz, 1H), 7.40 (d, *J* = 7.5 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 15.6, 21.6, 39.5, 41.7, 43.8, 89.4, 122.2, 122.8, 126.0 (2C), 128.1, 130.4 (2C), 130.9, 131.9, 136.6, 142.7, 148.1, 167.8.

*8-Methyl-5-(4-methylsulfanylbenzyl)-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **10b**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.33 (s, 3H), 2.35 (s, 3H), 2.81-2.98 (m, 2H), 3.09 (d, *J* = 14.0 Hz, 1H), 3.32 (d, *J* = 14.0 Hz, 1H), 3.95-4.15 (m, 2H), 4.20 (bs, 1H, D_2O), 6.73 (d, *J* = 8.3 Hz, 2H), 6.93 (d, *J* = 8.3 Hz, 2H), 7.12 (d, *J* = 7.2 Hz, 1H), 7.20 (d, *J* = 7.2 Hz, 1H), 7.32 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 15.6, 21.4, 39.5, 41.7, 43.8, 89.4, 122.8, 123.3, 126.0 (2C), 128.1, 130.4 (2C), 130.9, 132.8, 136.6, 139.0, 144.9, 167.8.

*4.1.2.11. 7(8)-Methyl-5-(4-methylsulfinylbenzyl)-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **11**. Yield: 89%. Oil. IR (NaCl): ν_{max} 3422, 2924, 1685, 1619, 1406, 1303, 1148, 1088, 959, 821, 733 cm^{-1}. HRMS (ESI^+) calcd. for $C_{19}H_{21}N_2O_2S$ $[M+H]^+$: 341.1318, found 341.1322.

*7-Methyl-5-(4-methylsulfinylbenzyl)-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **11a**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.36 (s, 3H), 2.57 (s, 3H), 2.99-3.18 (m, 2H), 3.13 (d, *J* = 13.6 Hz, 1H), 3.40 (d, *J* = 13.6 Hz, 1H), 4.02-4.12 (m, 2H), 6.98 (d, *J* = 2.0 Hz, 1H), 7.12 (dd, *J* = 8.0, 2.0 Hz, 1H),

7.25 (d, *J* = 8.0 Hz, 1H), 7.28 (d, *J* = 8.0 Hz, 2H), 7.33 (d, *J* = 8.0 Hz, 2H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.4, 22.0, 39.1, 41.8, 43.7, 88.8, 122.7, 122.8, 126.9, 123.3 (2C), 132.9 (2C), 129.9, 130.8, 143.7, 143.8, 148.0, 167.6.

*8-Methyl-5-(4-methylsulfinyl)benzyl-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **11b**. ^{1}H NMR (200 MHz, $CDCl_3$): 2.27 (s, 3H), 2.57 (s, 3H), 2.83-3.02 (m, 2H), 3.13 (d, *J* = 13.6 Hz, 1H), 3.40 (d, *J* = 13.6 Hz, 1H), 4.02-4.12 (m, 2H), 7.00 (d, *J* = 7.6, 1H), 7.18 (d, *J* = 7.6 Hz, 1H), 7.21 (bs, 1H), 7.28 (d, *J* = 8.0 Hz, 2H), 7.33 (d, *J* = 8.0 Hz, 2H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.4, 22.0, 39.1, 41.8, 43.7, 88.8, 122.0, 123.1, 123.3 (2C), 127.2, 130.8, 131.1, 132.9 (2C), 138.7, 143.8, 144.8, 167.6.

*4.1.2.12. 7(8)-Methyl-5-(4-methylsulfonylbenzyl)-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **12**. Yield: 91%. Oil. IR (NaCl): ν_{max} 3372, 2924, 1685, 1597, 1409, 1302, 1149, 1090, 959, 826, 729 cm^{-1}. HRMS (ESI$^+$) calcd. for $C_{19}H_{21}N_2O_3S$ $[M+H]^+$: 357.1267, found 357. 1267.

*7-Methyl-5-(4-(methylsulfonyl)benzyl)-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **12a**. ^{1}H NMR (200 MHz, $CDCl_3$): 2.37 (s, 3H), 2.98 (s, 3H), 3.00-3.18 (m, 2H), 3.08 (d, *J* = 13.6 Hz, 1H), 3.39 (d, *J* = 13.6 Hz, 1H), 4.00-4.12 (m, 2H), 7.01 (d, *J* = 8.0 Hz, 2H), 7.20 (bs, 1H), 7.26 (d, *J* = 7.6 Hz, 1H), 7.40 (d, *J* = 7.6 Hz, 1H), 7.60 (d, *J* = 8.0 Hz, 2H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 22.7, 45.1, 39.7, 42.5, 45.2, 89.2, 122.6, 124.1, 127.5, 127.6 (2C), 131.7 (2C), 131.6, 133.7, 140.0, 142.5, 145.3, 168.2.

*8-Methyl-5-(4-(methylsulfonyl)benzyl)-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **12b**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.32 (s, 3H), 2.98 (s, 3H), 3.00-3.18 (m, 2H), 3.08 (d, *J* = 13.6 Hz, 1H), 3.39 (d, *J* = 13.6 Hz, 1H), 4.00-4.12 (m, 2H), 7.03 (d, *J* = 8.0 Hz, 2H), 7.13 (d, *J* = 7.6 Hz, 1H), 7.16 (d, *J* = 7.6 Hz, 1H), 7.33 (bs, 1H), 7.61 (d, *J* = 8.0 Hz, 2H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 22.0, 45.1, 39.7, 42.5, 45.2, 89.2, 123.3, 123.7, 127.6 (2C), 130.7, 131.6, 131.7 (2C), 131.3, 139.4, 140.0, 143.6, 168.2.

*4.1.2.13. 5-(2,4-Difluorobenzyl)-7(8)-methyl-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **13**. Yield: 65%. Oil. IR (NaCl): ν_{max} 3360, 2930, 1680, 1620, 1490, 1249, 1080, 820, 720, 760 cm^{-1}. HRMS (ESI$^+$) calcd. for $C_{18}H_{17}F_2N_2O$ $[M+H]^+$: 315.1309, found 315.1305.

*5-(2,4-Difluorobenzyl)-7-methyl-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **13a**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.44 (s, 3H), 2.86-3.18 (m, 2H), 3.28 (d, *J* = 14.0 Hz, 1H), 3.39 (d, *J* = 14.0 Hz, 1H), 4.10-4.17 (m, 2H), 6.56-6.67 (m, 3H), 7.27 (bs, 1H), 7.29 (d, *J* = 7.5 Hz, 1H), 7.47 (d, *J* = 7.5 Hz, 1H), ^{13}C NMR (50 MHz, $CDCl_3$): δ 22.0, 36.0, 39.1, 41.6, 88.8, 103.5 (t, *J* =25.7 Hz), 110.7 (d, *J* = 20.2 Hz), 118.0 (d, *J* = 14.7 Hz), 123.2, 128.1, 129.9, 130.8, 132.3 (m), 142.8, 148.0, 160.5 (dd, *J* = 247.7, 12.9 Hz), 161.5 (dd, *J* = 247.7, 12.9 Hz), 167.6.

*5-(2,4-Difluorobenzyl)-8-methyl-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **13b**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.38 (s, 3H), 2.86-3.18 (m, 2H), 3.28 (d, *J* = 14.0 Hz, 1H), 3.35 (d, *J* = 14.0 Hz, 1H), 4.10-4.17 (m, 2H), 6.56-6.67 (m, 3H), 7.18 (d, *J* = 7.9 Hz, 1H), 7.32 (s, 1H), 7.37 (d, *J* = 7.9 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.4, 36.0, 39.1, 41.6, 88.8, 103.5 (t, *J* =25.7 Hz), 110.7 (d, *J* = 20.2 Hz), 118.0 (d, *J* = 14.7 Hz), 122.0, 123.2, 130.9, 132.3 (m), 139.2, 142.8, 144.9, 160.5 (dd, *J* = 247.7 Hz, 12.9), 161.5 (dd, *J* = 247.7 Hz, 12.9), 167.6.

*4.1.2.14. 5-(2,4-Dichlorobenzyl)-7(8)-methyl-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **14**.

Yield: 47%. Oil; IR (NaCl): ν_{max} 3354, 2926, 1682, 1621, 1473, 1080, 1051, 831 cm^{-1}. HRMS (ESI^+) calcd. for $C_{18}H_{17}Cl_2N_2O$ $[M+H]^+$: 347.0712, found 347. 0718.

*5-(2,4-Dichlorobenzyl)-7-methyl-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **14a**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.34 (s, 3H), 2.85-3.15 (m, 2H), 3.25 (d, *J* = 14.1 Hz, 1H), 3.40 (bs, 1H, D_2O), 3.40 (d, *J* = 14.1 Hz, 1H), 4.02-4.10 (m, 2H), 6.86 (d, *J* = 8.3 Hz, 1H), 6.94 (dd, *J* = 8.3, 1.8 Hz, 1H), 7.11 (bs, 1H), 7.17 (d, *J* = 8.3 Hz, 1H), 7.19 (s, 1H), 7.46 (d, *J* = 8.3 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 22.0, 39.1, 40.4, 41.9, 88.6, 123.0, 126.8 (2C), 128.0, 129.3, 130.0, 130.8, 132.4, 133.3, 135.6, 142.8, 148.3, 167.7.

*5-(2,4-Dichlorobenzyl)-8-methyl-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **14b**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.32 (s, 3H), 2.85-3.13 (m, 2H), 3.19 (d, *J* = 14.1 Hz, 1H), 3.40 (bs, 1H, D_2O), 3.47 (d, *J* = 14.1 Hz, 1H), 4.02-4.13 (m, 2H), 6.86 (d, *J* = 8.3 Hz, 1H), 6.94 (dd, *J* = 8.3, 1.8 Hz, 1H), 7.11 (bs, 1H), 7.16 (d, *J* = 7.0 Hz, 1H), 7.22 (d, *J* = 7.0 Hz, 1H), 7.39 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.5, 39.1, 40.4, 41.9, 88.6, 122.3, 123.4, 126.8, 128.0, 129.3, 132.1, 132.4, 132.9, 133.3, 135.6, 139.2, 145.2, 167.7.

*4.1.2.15. 5-(3,4-Dichlorobenzyl)-7(8)-methyl-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **15**.

Yield: 39%. Oil. IR (NaCl): ν_{max} 3293, 2926, 1684, 1620, 1442, 1081, 909, 810, 733 cm^{-1}. HRMS (ESI^+) calcd. for $C_{18}H_{17}Cl_2N_2O$ $[M+H]^+$: 347.0712, found 347.0707.

*5-(3,4-Dichlorobenzyl)-7-methyl-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **15a**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.38 (s, 3H), 2.89-2.98 (m, 2H), 3.00 (d, *J* = 13.8 Hz, 1H), 3.30 (d, *J* = 13.8 Hz, 1H), 4.00 (sa, 1H, D_2O), 3.96-4.05 (m, 2H), 6.62 (dd, *J* = 8.0, 2.2 Hz, 1H), 6.93 (d, *J* = 2.2 Hz, 1H), 7.12 (d, *J* = 8.0 Hz, 1H), 7.13 (s, 1H), 7.15 (d, *J* = 7.8 Hz, 1H), 7.42 (d, *J* = 7.8 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 19.3, 36.7, 38.9, 40.8, 86.2, 120.1, 120.3, 125.4, 126.7, 127.1, 127.4, 128.2, 129.1, 129.3, 132.8, 140.3, 145.0, 165.0.

*5-(3,4-Dichlorobenzyl)-8-methyl-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **15b**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.34 (s, 3H), 2.98-2.89 (m, 2H), 3.00 (d, *J* = 13.8 Hz, 1H), 3.30 (d, *J* = 13.8 Hz, 1H), 4.00 (sa, 1H, D_2O), 3.96-4.05 (m, 2H), 6.62 (dd, *J* = 8.0, 2.2 Hz, 1H), 6.93 (d, *J* = 2.2 Hz, 1H),

7.12 (d, *J* = 8.0 Hz, 1H), 7.17 (d, *J* = 7.8 Hz, 1H), 7.27 (d, *J* = 7.8 Hz, 1H), 7.35 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 18.7, 36.7, 38.9, 40.8, 86.3, 119.4, 120.7, 125.4, 126.6, 127.1, 128.2, 129.0, 129.3, 130.3, 132.8, 136.7, 141.8, 165.0.

*4.1.2.16. 5-(3,4-Methylendioxybenzyl)-7(8)-methyl-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol,* **16**. Yield: 39%. Oil. IR (NaCl): ν_{max} 3356, 2923, 1683, 1620, 1493, 1247, 1039, 909, 731 cm^{-1}. HRMS (ESI$^+$) calcd. for $C_{19}H_{19}N_2O_3$ $[M+H]^+$: 323.1396, found 323.1390.

*5-(3,4-Methylendioxybenzyl)-7-methyl-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **16a**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.43 (s, 3H), 2.93-3.10 (m, 2H), 3.13 (d, *J* = 14.0 Hz, 1H), 3.33 (d, *J* = 14.0 Hz, 1H), 4.02-4.11 (m, 2H), 3.72 (sa, 1H, D_2O), 5.83 (s, 2H), 6.27 (d, *J* = 2.2 Hz, 1H), 6.32 (dd, *J* = 8.1, 2.2 Hz, 1H), 6.52 (d, *J* = 8.1Hz, 1H), 7.30 (bs, 1H), 7.25 (d, *J* = 7.5 Hz, 1H), 7.48 (d, *J* = 7.5 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 22.0, 39.6, 41.6, 43.8, 89.7, 100.8, 107.7, 110.2, 122.7, 122.9, 123.2, 128.7, 129.9, 130.9, 142.7, 146.3, 147.1, 148.1, 167.8.

*5-(3,4-Methylendioxybenzyl)-8-methyl-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **16b**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.37 (s, 3H), 3.10-2.93 (m, 2H), 3.13 (d, *J* = 14.0 Hz, 1H), 3.33 (d, *J* = 14.0 Hz, 1H), 4.02-4.11 (m, 2H), 3.72 (bs, 1H, D_2O), 5.83 (s, 2H), 6.27 (d, *J* = 2.2 Hz, 1H), 6.32 (dd, *J* = 8.1, 2.2 Hz, 1H), 6.52 (d, *J* = 8.1 Hz, 1H), 7.18 (d, *J* = 7.5 Hz, 1H), 7.22 (d, *J* = 7.5 Hz, 1H), 7.41 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.4, 39.6, 41.6, 43.8, 89.6, 100.8, 107.7, 110.2, 122.2, 123.2, 128.3, 129.9, 130.9, 132.8, 139.0, 144.9, 147.1, 148.1, 167.8.

*4.1.2.17. 5-(3,4-Dimethoxybenzyl)-7(8)-methyl-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **17**. Yield: 58%. Oil. IR (NaCl): ν_{max} 3360, 2932, 1683, 1616, 1515, 1263, 1147, 1028, 905, 731 cm^{-1}. HRMS (ESI$^+$) calcd. for $C_{20}H_{23}N_2O_3$ $[M+H]^+$: 339.1703, found 339.1683.

*5-(3,4-Dimethoxybenzyl)-7-methyl-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol,* **17a**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.44 (s, 3H), 3.03-2.89 (m, 2H), 3.19 (d, *J* = 14.0 Hz, 1H), 3.35 (d, *J* = 14.0 Hz, 1H), 3.58 (s, 3H), 3.77 (s, 3H), 4.05-4.14 (m, 2H), 6.24 (d, *J* = 2.2 Hz, 1H), 6.46 (dd, *J* = 8.3, 2.1 Hz, 1H), 6.61 (d, *J* = 8.3 Hz, 1H), 7.32 (bs, 1H), 7.35 (m, 1H), 7.48 (d, *J* = 7.5 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): 21.9, 39.6, 41.7, 43.8, 55.5, 55.7, 89.6, 110.6, 113.0, 122.2, 122.8, 123.2, 127.5, 128.5, 129.7, 142.5, 147.7, 148.1, 148.3, 167.6.

*5-(3,4-Dimethoxybenzyl)-8-methyl-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol,* **17b**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.37 (s, 3H), 3.03-2.89 (m, 2H), 3.16 (d, *J* = 14.0 Hz, 1H), 3.34 (d, *J* = 14.0 Hz, 1H), 3.58 (s, 3H), 3.77 (s, 3H), 4.05-4.14 (m, 2H), 6.24 (d, *J* = 2.1 Hz, 1H), 6.45 (dd, *J* = 8.2, 2.1 Hz, 1H), 6.61 (d, *J* = 8.2 Hz, 1H), 7.19 (bd, *J* = 7.9 Hz, 1H), 7.33 (d, *J* = 7.9 Hz, 1H), 7.40 (bs, 1H), ^{13}C NMR (50 MHz, $CDCl_3$): 21.3, 39.6, 41.7, 43.8, 55.5, 55.7, 89.4, 110.6, 113.0, 122.2, 122.8, 123.2, 128.6, 129.7, 132.6, 138.9, 145.2, 148.1, 148.3, 167.6.

*4.1.2.18. 5-(3,4,5-Trimethoxybenzyl)-7(8)-methyl-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **18**. Yield: 50%. Oil. IR (NaCl): ν_{max} 3357, 2928, 1681, 1590, 1457, 1241, 1185, 836, 784 cm^{-1}. HRMS (ESI^+) calcd. for $C_{21}H_{25}N_2O_4$ $[M+H]^+$: 369.1809, found 369.1789.

*5-(3,4,5-Trimethoxybenzyl)-7-methyl-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **18a**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.40 (s, 3H), 2.80-3.07 (m, 2H), 3.13 (d, *J* = 14.0 Hz, 1H), 3.32 (d, *J* = 14.0 Hz, 1H), 3.59 (s, 6H), 3.72 (s, 3H), 3.98-4.10 (m, 2H), 6.01 (s, 2H), 7.33 (d, *J* = 7.5 Hz, 1H), 7.38 (bs, 1H), 7.46 (d, *J* = 7.5 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.9, 39.4, 41.7, 44.6, 55.9 (2C), 60.8, 89.3, 107.1 (2C), 122.2, 128.5, 129.7 (2C), 131.2, 136.7, 142.6, 148.4, 152.5 (2C), 167.7.

*5-(3,4,5-Trimethoxybenzyl)-8-methyl-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **18b**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.35 (s, 3H), 2.80-3.07 (m, 2H), 3.13 (d, *J* = 14.0 Hz, 1H), 3.11 (d, *J* = 14.0 Hz, 1H), 3.59 (s, 6H), 3.72 (s, 3H), 3.98-4.10 (m, 2H), 6.01 (s, 2H), 7.16 (bd, *J* = 7.9 Hz, 1H), 7.36 (d, *J* = 7.9 Hz 1H), 7.38 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.3, 39.4, 41.7, 44.6, 55.9 (2C), 60.8, 89.2, 107.1 (2C), 122.8, 123.3, 130.7, 131.2, 132.6, 136.7, 139.1, 145.3, 152.5 (2C), 167.7.

*4.1.2.19. 7(8)-Methyl-5-(naphthalen-1-ylmethyl)-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **19**. Yield: 96%. Oil. IR (NaCl): ν_{max} 3294, 2927, 1685, 1620, 1455, 1396, 1273, 1084, 732 cm^{-1}. HRMS (ESI^+) calcd. for $C_{22}H_{21}N_2O$ $[M+H]^+$: 329.1648, found 329.1654.

*7-Methyl-5-(naphthalen-1-ylmethyl)-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **19a**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.33 (s, 3H), 2.84 (m, 2H), 3.61 (d, *J* = 13.8 Hz, 1H), 3.79 (d, *J* = 13.8 Hz, 1H), 3.58-3.89 (m, 2H), 6.96 (m, 2H), 7.30 (s, 1H), 7.38 (m, 2H), 7.49 (d, *J* = 7.9 Hz, 1H), 7.71 (d, *J* = 7.9 Hz, 1H), 7.77 (m, 1H), 7.79 (m, 1H), 7.93 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.7, 39.0, 41.1, 42.0, 89.0, 122.8, 123.4, 124.4, 125.1, 125.4, 125.6, 127.6, 127.8, 128.6, 128.9, 129.6, 132.6, 132.9, 133.7, 142.5, 149.0, 167.8.

*8-Methyl-5-(naphthalen-1-ylmethyl)-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **19b**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.21 (s, 3H), 2.84 (m, 2H), 3.61 (d, *J* = 13.8 Hz, 1H), 3.79 (d, *J* = 13.8 Hz, 1H), 3.58-3.89 (m, 2H), 6.96 (m, 2H), 7.27 (d, *J* = 7.0 Hz, 1H), 7.36 (d, *J* = 7.0 Hz, 1H), 7.38 (m, 2H), 7.55 (s, 1H), 7.77 (m, 1H), 7.79 (m, 1H), 7.93 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.3, 39.0, 41.1, 42.0, 89.0, 122.5, 123.4, 124.4, 125.1, 125.4, 125.6, 127.6, 127.8, 128.6, 129.6, 132.0, 132.6, 132.9, 133.7, 138.9, 146.2, 167.8.

*4.1.2.20. 7(8)-Methyl-5-(naphthalen-2-ylmethyl)-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **20**. Yield: 96%. Oil. IR (NaCl): ν_{max} 3290, 2926, 1684, 1618, 1396, 1078, 825, 779, 742 cm^{-1}. HRMS (ESI^+) calcd. for $C_{22}H_{21}N_2O$ $[M+H]^+$: 329.1654, found 329.1655.

*7-Methyl-5-(naphthalen-2-ylmethyl)-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **20a**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.32 (s, 3H), 3.09-3.21 (m, 2H), 3.28 (d, *J* = 13.5 Hz, 1H), 3.52 (d, *J* = 13.5 Hz, 1H), 3.93-4.00 (m, 2H), 4.28 (s, 1H, D_2O), 7.31 (s, 1H), 7.48 (d, *J* = 8.0 Hz, 1H), 7.55-6.98 (m, 7H), 7.70 (d, *J* = 8.0 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 22.0, 39.8, 41.6, 44.4, 89.8, 122.7, 125.6, 125.9, 127.4, 127.6, 128.3, 129.0, 129.9, 130.0, 130.9, 131.0, 132.8 (2C), 133.1, 142.6, 148.1, 167.8.

*8-Methyl-5-(naphthalen-2-ylmethyl)-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **20b**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.26 (s, 3H), 2.77-2.82 (m, 2H), 3.24 (d, *J* = 13.5 Hz, 1H), 3.52 (d, *J* = 13.5 Hz, 1H), 3.93-4.00 (m, 2H), 7.29 (d, *J* = 8.0 Hz, 1H), 7.38 (d, *J* = 8.0 Hz, 1H), 7.52 (s, 1H), 7.55-6.98 (m, 7H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.3, 39.8, 41.6, 44.4, 89.7, 122.4, 123.1, 125.6, 127.4, 127.6, 128.3, 129.0, 129.9, 130.9, 131.0, 132.3, 132.8 (2C), 133.1, 139.0, 144.9, 167.8.

*4.1.2.21. 5-(4-Chlorobenzyl)-6(9)-nitro-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **21**.

Yield: 60%. Oil. IR (NaCl): ν_{max} 3422, 2927, 1701, 1622, 1537, 1358, 1080, 1015, 815, 725 cm^{-1}. HRMS (ESI$^+$) calcd. for $C_{17}H_{15}ClN_3O_3$ $[M+H]^+$: 344.0796, found 344.0790.

*5-(4-Chlorobenzyl)-6-nitro-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **21a**.

^{1}H NMR (400 MHz, $CDCl_3$): δ 3.31-3.50 (m, 2H), 3.33 (d, *J* = 14.0 Hz, 1H), 3.41 (d, *J* = 14.0 Hz, 1H), 3.93-4.12 (m, 2H), 6.62 (d, *J* = 8.0 Hz, 2H), 7.06 (d, *J* = 8.0 Hz, 2H), 7.52 (dd, *J* = 8.0, 7.9 Hz, 1H), 7.61 (d, *J* = 7.9, 1H), 8.10 (d, *J* = 8.0 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 38.9, 41.1, 42.7, 92.1, 123.8, 126.9, 127.7, 128.4 (4C), 131.4, 132.6, 134.2, 140.0, 149.6, 164.8.

*5-(4-Chlorobenzyl)-9-nitro-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **21b**.

^{1}H NMR (400 MHz, $CDCl_3$): δ 3.07-3.10 (m, 2H), 3.33 (d, *J* = 14.0 Hz, 1H), 3.41 (d, *J* = 14.0 Hz, 1H), 3.93-4.12 (m, 2H), 6.78 (d, *J* = 7.6 Hz, 2H), 6.92 (d, *J* = 7.6 Hz, 2H), 7.36 (d, *J* = 7.2, Hz, 1H), 7.46 (dd, *J* = 7.2, 7.3, 1H), 7.71 (d, *J* = 7.3 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 38.8, 40.3, 41.1, 89.6, 122.6, 123.8, 128.4 (4C), 130.6, 131.4, 132.9, 133.2, 144.7, 145.5, 163.1.

*4.1.2.22. 5-(4-Chlorobenzyl)-7(8)-(hydroxymethyl)-3,5-dihydro-2*H-*imidazo*[*2,1*-a*]isoindol-5-ol*, **22**.

Yield: 87%. Oil. IR (NaCl): ν_{max} 3368, 2925, 1677, 1620, 1422, 1285, 1210, 1015, 815, 812, 720 cm^{-1}. HRMS (ESI$^+$) calcd. for $C_{18}H_{18}ClN_2O_2$ $[M+H]^+$: 329.1051, found 329.1041.

*5-(4-Chlorobenzyl)-7-(hydroxymethyl)-3,5-dihydro-2*H-*imidazo*[*2,1*-a*]isoindol-5-ol*, **22a**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.89-3.13 (m, 2H), 3.14 (d, *J* = 14.0 Hz, 1H), 3.29 (d, *J* = 14.0 Hz, 1H), 3.89-4.10 (m, 2H), 4.57 (s, 2H), 6.99 (d, *J* = 8.4 Hz, 2H), 7.01 (d, *J* = 8.4 Hz, 2H), 7.19 (d, *J* = 8.0 Hz, 1H), 7.28 (s, 1H), 7.40 (d, *J* = 8.0 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 39.2, 41.2, 42.7, 63.5, 89.3, 119.7, 122.4, 126.8, 127.5 (2C), 130.2, 130.8, 130.6 (2C), 132.8, 142.4, 146.7, 167.2.

*5-(4-Chlorobenzyl)-8-(hydroxymethyl)-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol,* **22b**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.79-3.35 (m, 2H), 3.14 (d, *J* = 14.0 Hz, 1H), 3.29 (d, *J* = 14.0 Hz, 1H), 3.85-4.10 (m, 2H), 4.49 (s, 2H), 6.73 (d, *J* = 8.4 Hz, 2H), 6.68 (d, *J* = 8.4 Hz, 2H), 7.16 (d, *J* = 8.0 Hz, 2H), 7.33 (s, 1H), 7.34 (d, *J* = 8.0 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 39.1, 41.2, 42.6, 63.4, 89.2, 120.6, 121.8, 127.5 (2C), 130.1, 132.1, 130.6 (2C), 132.8, 145.6 (2C), 167.1.

*4.1.2.23. 5-(4-Chlorobenzyl)-5-hydroxy-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindole-7(8)-carbaldehyde,* **23**.

Yield: 79%. Oil. IR (NaCl): ν_{max} 3296, 2928, 1704, 1682, 1620, 1347, 1088, 815, 779, 732 cm^{-1}. HRMS (ESI$^+$) calcd. for $C_{18}H_{16}ClN_2O_2$ $[M+H]^+$: 327.0900, found 327.0907.

*5-(4-Chlorobenzyl)-5-hydroxy-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindole-7-carbaldehyde,* **23a**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.12 (d, *J* = 14.0 Hz, 1H), 3.33-2.80 (m, 2H), 3.39 (d, *J* = 14.0 Hz, 1H), 4.15-3.96 (m, 2H), 6.86 (d, *J* = 8.4 Hz, 2H), 7.14 (d, *J* = 8.4 Hz, 2H), 7.29 (d, *J* = 7.9 Hz, 1H), 7.31 (s, 1H), 7.51 (d, *J* = 7.9 Hz, 1H), 10.10 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 39.7, 40.9, 43.5, 90.0, 128.1 (2C), 128.9, 129.6, 130.0, 130.9, 131.2 (2C), 131.7, 133.4, 139.7, 143.8, 168.0, 190.8.

*5-(4-Chlorobenzyl)-5-hydroxy-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindole-8-carbaldehyde,* **23b**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.12 (d, *J* = 14.0 Hz, 1H), 3.33-2.80 (m, 2H), 3.39 (d, *J* = 14.0 Hz, 1H), 4.15-3.96 (m, 2H), 6.88 (d, *J* = 8.4 Hz, 2H), 7.16 (d, *J* = 8.4 Hz, 2H), 7.17 (s, 1H), 7.19 (d, *J* = 8.1 Hz, 1H), 7.58 (d, *J* = 8.1 Hz, 1H), 10.17 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 39.7, 40.9, 43.5, 90.0, 128.1 (2C), 128.6, 128.9, 130.9, 131.2 (2C), 131.7, 132.0, 133.4, 139.6, 143.5, 168.0, 190.6.

*4.1.2.24. 5-(4-Chlorobenzyl)-7(8)-nitro-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **24**.

Yield: 85%. Oil. IR (NaCl): ν_{max} 3360, 2925, 1699, 1533, 1430,1348, 1235 1112, 1086, 903, 813, 723 cm^{-1}. HRMS (ESI$^+$) calcd. for $C_{17}H_{15}ClN_3O_3$ $[M+H]^+$: 344.0796, found 344.0788.

*5-(4-Chlorobenzyl)-7-nitro-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol,* **24a**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.92-3.13 (m, 2H), 3.27 (d, *J* = 14.1 Hz, 1H), 3.41 (d, *J* = 14.1 Hz, 1H), 4.08-4.21 (m, 2H), 6.82 (d, *J* = 8.0 Hz, 2H), 7.09 (d, *J* = 8.0 Hz, 2H), 7.72 (d, *J* = 8.2 Hz, 1H), 8.26 (bs, 1H), 8.36 (d, *J* = 8.2 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 38.9, 42.0, 43.6, 89.1, 117.9, 124.1, 128.3 (2C), 127.0, 128.4, 131.1 (2C), 132.2, 133.1, 144.8, 149.0, 165.2.

*5-(4-Chlorobenzyl)-8-nitro-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol,* **24b**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.92-3.13 (m, 2H), 3.26 (d, *J* = 14.1 Hz, 1H), 3.41 (d, *J* = 14.1 Hz, 1H), 4.08-4.21 (m, 2H), 6.78 (d, *J* = 7.9 Hz, 2H), 7.07 (d, *J* = 7.9 Hz, 2H), 7.49 (d, *J* = 8.2 Hz, 1H), 8.24 (d, *J* = 8.2 Hz, 1H), 8.41 (bs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 38.9, 41.8, 43.6, 89.0, 118.6, 123.6, 124.6, 128.3 (2C), 128.4, 131.1 (2C), 132.2, 132.6, 141.6, 150.2, 165.2.

*4.1.2.25. 6,9-Dichloro-5-(2-chlorobenzyl)-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **25**.

Yield: 92%. Oil. IR (NaCl): ν_{max} 3196, 2925, 1687, 1612, 1396, 1235, 1088, 895, 736 cm^{-1}. HRMS (ESI^+) calcd. for $C_{17}H_{14}Cl_3N_2O$ $[M+H]^+$: 367.0166, found 367.0160. ^1H NMR (400 MHz, $CDCl_3$): δ 2.82-3.13 (m, 2H), 3.63 (d, *J* = 14.4 Hz, 1H), 3.78 (d, *J* = 14.4 Hz, 1H), 3.93-3.98 (m, 2H), 4.24 (bs, 1H, D_2O), 6.76 (dd, *J* = 7.2, 1.2, 1H), 6.87 (ddd, *J* = 8.0, 7.2, 1.6, 1H), 6.98 (ddd, *J* = 8.4, 8.0, 1.2, 1H), 7.16 (dd, *J* = 8.4, 1.6, 1H), 7.21 (d, *J* = 8.4 Hz, 1H), 7.37 (d, *J* = 8.4 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 37.9, 38.3, 42.3, 88.6, 126.3, 128.0, 128.4, 128.8, 129.3, 129.7, 131.4, 132.0, 132.4, 133.9, 134.7, 146.2, 163.8.

*4.1.2.26. 6,9-Dichloro-5-(4-chlorobenzyl)-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **26**.

Yield: 91%. Oil. IR (NaCl): ν_{max} 3234, 2930, 1685, 1598, 1376, 1225, 1093, 815, 732 cm^{-1}. HRMS (ESI^+) calcd. for $C_{17}H_{14}Cl_3N_2O$ $[M+H]^+$: 367.0172, found 367.0173. ^1H NMR (400 MHz, $CDCl_3$): δ 2.96-3.05 (m, 2H), 3.21 (d, *J* = 14.0 Hz, 1H), 3.76 (d, *J* = 14.0 Hz, 1H), 4.04-4.08 (m, 2H), 4.32 (bs, 1H, D_2O), 6.70 (d, *J* = 8.8 Hz, 2H), 6.97 (d, *J* = 8.8 Hz, 2H), 7.33 (d, *J* = 8.8 Hz, 2H), 7.36 (d, *J* = 8.8 Hz, 1H), 7.43 (d, *J* = 7.9 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 38.4, 40.1, 41.4, 89.1, 127.6, 128.4 (2C), 129.4, 130.4 (2C), 132.1, 132.8, 132.9, 133.9, 145.2, 164.1.

*4.1.2.27. 6,9-Dichloro-5-(4-methylsulfanylbenzyl)-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **27**.

Yield: 95%. Oil. IR (NaCl): ν_{max} 3367, 2923, 1700, 1594, 1459, 1161, 1089, 822, 734 cm^{-1}. HRMS (ESI^+) calcd. for $C_{18}H_{17}Cl_2N_2OS$ $[M+H]^+$: 379.0433, found 379.0411. ^1H NMR (400 MHz, $CDCl_3$): δ 2.34 (s, 3H), 2.97-3.05 (m, 2H), 3.24 (d, *J* = 14.0 Hz, 1H), 3.80 (d, *J* = 14.0 Hz, 1H), 4.07-4.12 (m, 2H), 6.73 (d, *J* = 8.0 Hz, 2H), 6.91 (d, *J* = 8.0 Hz, 2H), 7.19 (d, *J* = 8.4 Hz, 1H), 7.34 (d, *J* = 8.0 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.1, 38.4, 40.2, 41.5, 89.2, 126.2 (2C), 127.6 (2C), 128.8, 129.3, 129.5, 131.0, 131.9, 133.8, 137.0, 145.5, 164.1.

*4.1.2.28. 7,8-Dichloro-5-(4-chlorobenzyl)-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **28**.

Yield: 55%. Oil. IR (NaCl): ν_{max} 3297, 2929, 1693, 1604, 1406, 1297, 1088, 895, 814, 732 cm^{-1}. HRMS (ESI^+) calcd. for $C_{17}H_{14}Cl_3N_2O$ $[M+H]^+$: 367.0172, found 367.0143. ^1H NMR (200 MHz, $CDCl_3$): δ 2.83-3.09 (m, 2H), 3.09 (d, *J* = 14.0 Hz, 1H), 3.31 (d, *J* = 14.0 Hz, 1H), 3.99-4.06 (m, 2H), 4.33 (bs, 1H, D_2O), 6.78 (d, *J* = 8.2 Hz, 2H), 7.07 (d, *J* = 8.2 Hz, 2H), 7.39 (s, 1H), 7.58 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 39.0, 41.9, 43.7, 88.8, 124.7, 125.0, 128.4 (2C), 130.5, 131.3 (2C), 132.9, 133.1, 133.7, 136.5, 147.0, 165.4.

*4.1.2.29. 7,8-Dichloro-5-(4-methylsulfanylbenzyl)-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol*, **29**.

Yield: 92%. Oil. IR (NaCl): ν_{max} 3296, 2924, 1692, 1604, 1406, 1298, 1087, 896, 812, 732 cm^{-1}. HRMS (ESI^+) calcd. for $C_{18}H_{17}Cl_2N_2OS$ $[M+H]^+$: 379.0433, found 379.0403. ^1H NMR (200 MHz,

$CDCl_3$): δ 2.38 (s, 3H), 2.85-3.25 (m, 2H), 3.08 (d, *J* = 14.0 Hz, 1H), 3.31 (d, *J* = 14.0 Hz, 1H), 4.02-4.16 (m, 2H), 4.18 (bs, 1H, D_2O), 6.76 (d, *J* = 7.5 Hz, 2H), 6.99 (d, *J* = 7.5 Hz, 2H), 7.44 (s, 1H), 7.61 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 15.6, 39.3, 42.0, 43.7, 89.1, 124.8 (2C), 126.1 (2C), 130.4 (2C), 130.6, 131.0, 133.4, 136.2, 137.2, 147.1, 165.4.

*4.1.2.30. 5-(3,4-Dichlorobenzyl)-6(9)-nitro-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol,* **30**.

Yield: 89%. Oil. IR (NaCl): ν_{max} 3427, 2926, 1701, 1537, 1471, 1356, 1090, 823, 769, 725 cm^{-1}. HRMS (ESI$^+$) calcd. for $C_{17}H_{14}Cl_2N_3O_3$ $[M+H]^+$: 378.0407, found 378.0407.

*5-(3,4-Dichlorobenzyl)-6-nitro-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol,* **30a**.

^{1}H NMR (400 MHz, $CDCl_3$): δ 2.86-3.03 (m, 2H), 3.29 (d, *J* = 14.0 Hz, 1H), 3.92 (d, *J* = 14.0 Hz, 1H), 4.06-4.10 (m, 2H), 6.57 (d, *J* = 8.0 Hz, 1H), 6.84 (s, 1H), 7.05 (d, *J* = 8.0 Hz, 1H), 7.52 (dd, *J* = 7.6, 8.0 Hz, 1H), 7.88 (d, *J* = 7.6 Hz, 1H), 8.13 (d, *J* = 8.0 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 39.3, 42.4, 42.5, 91.8, 127.2, 128.4, 129.0, 130.8, 131.2, 131.8, 132.0, 132.7, 134.8, 135.7, 140.9, 145.4, 165.1.

*5-(3,4-Dichlorobenzyl)-9-nitro-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol,* **30b**.

^{1}H NMR (400 MHz, $CDCl_3$): δ 2.86-3.03 (m, 2H), 3.29 (d, *J* = 14.0 Hz, 1H), 4.06-4.10 (m, 2H), 4.16 (d, *J* = 14.0 Hz, 1H), 6.65 (d, *J* = 8.0 Hz, 1H), 7.07 (s, 1H), 7.25 (d, *J* = 8.0 Hz, 1H), 7.41 (d, *J* = 7.6 Hz, 1H), 7.60 (dd, *J* = 7.6, 7.7 Hz, 1H), 7.67 (d, *J* = 7.7 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 39.4, 42.5, 44.2, 88.7, 123.1, 124.3, 127.2, 130.1, 131.7, 132.7, 132.8, 133.5, 134.7, 135.5, 146.4, 150.8, 163.0.

*4.1.2.31. 5-(3,4-Dichlorobenzyl)-7(8)-nitro-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol,* **31**.

Yield: 91%. Oil; IR (NaCl): ν_{max} 3427, 2925, 1698, 1533, 1472, 1348, 1031, 902, 820, 728 cm^{-1}. HRMS (ESI$^+$) calcd. for $C_{17}H_{14}Cl_2N_3O_3$ $[M+H]^+$: 378.0407, found 378.0407.

*5-(3,4-Dichlorobenzyl)-7-nitro-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol,* **31a**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.84-2.90 (m, 2H), 3.15 (d, *J* = 14.0 Hz, 1H), 3.35 (d, *J* = 14.0 Hz, 1H), 4.05-4.16 (m, 2H), 4.47 (s, 1H, D_2O), 6.46 (d, *J* = 2.0, 1H), 6.63 (dd, *J* = 8.0, 2.0, 1H), 7.14 (d, *J* = 8.0 Hz, 1H), 7.68 (dd, *J* = 8.4, 1.6 Hz, 1H), 8.14 (d, *J* = 1.6 Hz, 1H), 8.30 (d, *J* = 8.4 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 39.0, 41.9, 43.4, 88.8, 117.9, 124.7, 127.1, 129.9, 130.1, 131.3, 131.8, 132.1, 132.2, 134.7, 135.8, 150.2, 165.1.

*5-(3,4-Dichlorobenzyl)-8-nitro-3,5-dihydro-2*H-*imidazo*[*2,1*-a]*isoindol-5-ol,* **31b**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.84-2.90 (m, 2H), 3.15 (d, *J* = 14.0 Hz, 1H), 3.35 (d, *J* = 14.0 Hz, 1H), 4.05-4.16 (m, 2H), 4.47 (s, 1H, D_2O), 6.67 (dd, *J* = 8.0, 1.6, 1H), 7.01 (d, *J* = 1.6, 1H), 7.15 (d, *J* = 8.0 Hz, 1H), 7.39 (d, *J* = 8.4 Hz, 1H), 8.19 (dd, *J* = 8.4, 2.0 Hz, 1H), 8.34 (d, *J* = 2.0 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 39.0, 42.1, 43.4, 88.8, 118.7, 123.7, 124.8, 129.9, 130.1, 131.3, 131.9, 132.1,

132.2, 134.6, 148.8, 153.0, 165.0.

4.2. Biological evaluation

4.2.1. Chemicals and drugs

Amphotericin B (AmB), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and phorbol-12-myristate-13-acetate (PMA), were purchased from Sigma Aldrich (St. Louis, MO). L-glutamine, and penicillin/streptomycin antibiotic were obtained from Gibco. Hygromycin B was purchased from Invitrogen (Carlsbad, CA).

4.2.2. *Cell lines culture and cytotoxicity assay*

Human myelomonocytic THP-1 cells were grown in RPMI-1640 supplemented with 10% heat-inactivated fetal bovine serum (hiFBS), 2 mM glutamine, 100 U/mL penicillin and 100 mg/mL streptomycin at 37 °C and 5% CO_2. THP-1 cells (3×10^4 cells per well in 96-well plates) were differentiated to macrophages by treatment with PMA (20 ng/mL, 48h), followed by 24 h of culture in fresh medium [43]. MRC5-SV2 cell line (SV40-transformed human lung fibroblast cell line) was cultured in DMEM medium supplemented with 10% hiFBS, 100 U/mL penicillin and 100 mg/mL streptomycin at 37 ºC and 5% CO_2. Cellular toxicity of all compounds on THP-1 and MRC-5 cells was determined using the colorimetric MTT-based assay after incubation at 37 °C for 72 h in presence of increasing concentrations of compounds [43]. To this end, cells were incubated with the corresponding concentration of compounds for 72 h at 37 °C. Afterwards, MTT reduction assay was carried out. Results were expressed as CC_{50} values (cytotoxicity concentration 50%, (Table 1), the concentration needed to reduce proliferation respect to untreated control cells. Assays were performed in three independent experiments done in triplicate.

4.2.3. *Leishmania culture and susceptibility assay*

Leishmania donovani MHOM/ET/67/HU3 line with luciferase gene integrated into the parasite genome [44] were grown at 28 °C in RPMI 1640-modified medium (Invitrogen) supplemented with 20% hiFBS (Invitrogen) with 100 mg/ml of hygromycin B. The 3-Luc strain of *L. donovani* parasites, expressing a cytoplasmic form of luciferase due to a mutation in its C-terminal tripeptide, was grown as above. The susceptibility of intracellular *L. donovani* amastigotes, as the clinical relevant forms of these parasites, to synthesized compounds was determined using the Luciferase Assay System Kit (Promega, Madison, Wis.) as previously described [45]. The luminescence output is indicative of the intracellular parasite growth. Briefly, macrophage-differentiated THP-1 cells

were infected at a macrophage/parasite ratio of 1:10 with stationary *L. donovani* promastigotes for 24 h at 35 °C and 5% CO_2. The non-phagocytized extracellular parasites were removed by washing with PBS (PBS: 130 mM NaCl, 1.2 mM KH_2PO_4, 8.1 mM Na_2HPO_4, 2.6 mM KCl, pH 7.0). Infected cell cultures were then incubated with the range of compound concentrations (from 0.62 to 40µM) at 37 °C for 72 h. Luminescence intensity was measured as indicative of the intracellular parasite growth after lysis with 0.1% Triton X-100 with an Infinite F200 microplate reader (Tecan Austria GmbH, Austria), according to the instructions provided by the supplier. The EC_{50} (concentration of compounds that reduce 50% the viability compared to untreated control cells) values were determined and compared with that for AmB as the reference drug. Selectivity indexes [SI] were calculated by dividing the CC_{50} values for the host cells THP-1 of a given compound by its respective EC_{50} for intracellular amastigotes. Assays were performed in three independent experiments, and samples were done in triplicate. AmB was used as standard anti-leishmanial agent. Assays were performed in three independent experiments done in triplicate.

Most potent compounds (EC_{50} = 1-3 µM) were also evaluated on *L. donovani* promastigotes. The viability of *L. donovani* promastigotes were measured by incubation of the promastigotes at 20 × 10^6 cells/mL in RPMI 1640 medium, devoid of hiFBS and phenol red (RPMI 1640- Ørred), for 4 h at 26 ºC. Afterwards, MTT was added (0.5 mg/mL final concentration), and its reduction by mitochondrial dehydrogenases allowed to proceed for 2 h, and the formazan precipitated solubilized by addition of 0.5% SDS final concentration. Absorbance was read at 595 nm in a Bio-Rad 680 Microplate Reader at 595 nm. Under these conditions, the MTT reduction measured the loss of viability of the parasites rather than the inhibition of proliferation [46]. Experiments were repeated at least twice. Samples were made by triplicate and IC_{50} (half-maximal inhibitory concentrations), the concentration of imidazoisoindolol that inhibited MTT reduction by 50%, was taken as a parameter for the loss of parasite viability. The mechanism of action for the most potent compounds **4**, **7**, **10**, **26**, **29** and **31** was also analysed on promastigote forms.

4.2.4. *Variation of bioenergetic parameters of* L. donovani *promastigotes by the imidazoisoindolol derivatives*

The modification of intracellular levels of ATP in living *L. donovani* parasites by the imidazoisoindolols were monitored using promastigotes of the *L. donovani* 3-luc strain. These promastigotes express a cytoplasmic form of luciferase inserted in the genome using a pLEXSY expression vector. The luminescence of these parasites in the presence of the caged luciferase substrate DMNPE-D-luciferin (D-Luciferin, 1-(4,5-dimethoxy-2-nitrophenyl)ethyl ester), is only limited by the concentration of intracellular ATP [47]. Promastigotes of the 3-Luc strain (20 × 10^6

cells/mL) were resuspended in RPMI 1640- Ored medium in the presence of 50 μM of DMNPE-luciferin; when a stable luminescence readout was reached, the compound at the corresponding concentration was added, and the luminescence monitored in a Thermoskan microplate reader (Thermo). Parasites with the respiratory chain inhibited by 1.5 μM 1,4-naphthoquinone were used as a control. The long term variation of intracellular ATP due to the imidazoisoindolols were measured by incubation of the parasites under identical conditions, except for the incubation time (4 h), and the fact that DMNPE-luciferin was added at the end of the incubation. The luminescence was recorded for 10 min after DMNPE-luciferin addition. In all cases the luminescence was referred respect to its value in untreated parasites.

4.2.5. *Determination of the electrochemical potential of mitochondrion of* L. donovani *lines after treatment with imidazoisoindolol derivatives*

Variation in the electrochemical potential of mitochondrion ($\Delta\Psi m$) was monitored by the intracellular accumulation of the probe Rh123 (Thermo-Fisher) whose accumulation inside the mitochondrion was driven by the $\Delta\Psi m$. To this end, *L. donovani* promastigotes (20×10^6 cells/mL) in RPMI 1640- Ored were first incubated with the compound at their respective concentration for 4 h, then, followed by a 10 min incubation with 0.5 μg/mL Rh123, washed for elimination of the non-incorporated Rh123, and the intracellular accumulation of the probe measured by flow cytometry in a Beckmann Coulter FC500 cytofluometer at 488 nm and 520 nm for excitation and emission wavelengths [46]. Parasites incubated with 10 mM KCN were used as controls.

4.2.6. *Determination of plasma membrane permeabilization in* L. donovani *lines by imidazoisoindolol derivatives.*

Plasma membrane permeabilization induced by the compounds was measured by the increase in fluorescence of the membrane-impermeable SYTOX-green after its intercalation with the intracellular nucleic acids. *L. donovani* promastigotes (20×10^6 parasites/mL) in RPMI 1640- Ored containing 1 μM SYTOX green were incubated with the corresponding compound. Changes in fluorescence were followed at 485 nm and 520 nm excitation and emission wavelengths, respectively in a Thermoskan microplate reader. Full permeabilized parasites were obtained by addition of 0.1 % Triton X-100 [46].

4.2.7. *Transmission electron microscopy analysis*.

Parasites (2×10^6 cells/mL) in complete growth medium were incubated with the compound **29** at its IC_{70} for 12 h; afterwards, parasites were processed for transmission electron microscopy as described previously [47]. Briefly, parasites were fixed with glutaraldehyde, contrasted with OsO_4, dehydrated under increasing concentrations of EtOH and embedded in Epon 812 epoxy resin (Tousimis).

Author contributions

All authors contributed to the writing of the manuscript.

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Appendix A. Supplementary data

^1H, ^{13}C NMR and HRMS spectra of representative compounds with EC_{50} values <10 μM against *L. donovani* amastigotes, and of intermediates **BP-1**, **BP-2** and **BP-3**.

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Highlights

1- Thirty one new imidazo[2,1-a]isoindolol (IIOL) derivatives have been synthesised, purified and tested *in vitro* against *L. donovani* amastigotes.

2- Twelve IIOL showed EC_{50} values against *L. donovani* amastigotes between 1.0 and 3.0 μM, with selectivity indexes ranging from 69.1 to 10.8, with respect to THP-1 cell line. Those compounds were also tested *in vitro* against *L. donovani* promastigotes and showed EC_{50} values between 2.1 and 13.3 μM.

3- Compounds **4**, **7, 10**, **26**, **29** and **31** were selected for elucidation of their leishmanicidal mechanism studies. Compound **29** interfered with the mitochondrial activity of the parasite, induce an early decrease of intracellular ATP levels, together with a partial destructuration of the plasma membrane.